Chapter

Pursuing the Perfect Performer of Fermented Beverages: GMMs vs. Microbial Consortium

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Abstract

Fermented beverages are widely diverse around the world and their quality is largely based on the organoleptic characteristics developed by the metabolism of the microorganisms present during fermentation. In order to achieve controllable processes in fermented beverages along with organoleptic complexity, two divergent approaches have been followed in terms of inoculum development: (1) the inoculation of multiple microorganisms, intending to promote synergism and favor organoleptic complexity derived from the metabolic diversity, and (2) the genetic modification of a single strain with the intention that it performs multiple functions. In this chapter, we discuss these divergent approaches, their achievements and perspectives.

Keywords: microbial consortium, genetic modified microorganism, biochemical changes, fermented beverages, organoleptic characteristics

1. Introduction

The induction of fermentation on raw materials provides new products with added nutrients and organoleptic complexity vastly appreciated by consumers. The changes in the components of the raw materials are mainly caused by the main and secondary metabolism of the microorganisms present during the fermentation processes. The microorganisms need carbon and nitrogen sources to obtain energy and structural blocks to maintain cell integrity and functions and to proliferate. However, some of the carbon and nitrogen are transformed and released to the medium as by-products of the metabolism which generate the characteristics of the fermented food. Spontaneous fermentation harbors complex evolving and diverse microbiota that provides organoleptic complexity, mainly in aromas and flavors. However, it is hard to control and usually derives in inconsistent and even defective products. This is why commercial starter cultures emerged, allowing a better control of fermentation. Nevertheless, some argue that commercial inoculation

leads to a loss of unique regional style. In these cases, flavors often considered superior are achieved, at the cost of consistency and occasional production losses. The microorganism core that causes the expected characteristics of several beverages has been studied widely, indicating the participation of multiple microorganisms through different stages of the fermentation. Two divergent approaches have been proposed to improve fermentation by the controlled inoculation of multiple microorganisms each causing different expected changes in the fermentation, or by the manipulation of the genome of single strains to perform multiple tasks by themselves. Both approaches have their strengths and weaknesses, and it seems that the next step is the combination of both strategies to provide a holistic solution.

2. Metabolism in fermented beverage processes

Fermentation is the metabolic process carried out by microorganisms to obtain energy by oxidizing carbohydrates in which the final electron acceptors are organic molecules rather than O_2 [1]. The catabolism of sugars results in the production of reduced pyridine nucleotides (nicotinamide adenine dinucleotide NADH); and to regenerate it in anaerobic conditions, pyruvate acts as the electron acceptor to reoxidate NADH [2]. The different fates of pyruvate are ethanol, lactic acid, or acetate, depending on the microorganism and environmental conditions [3].

2.1 Alcoholic fermentation

Alcoholic fermentation is the transformation of the sugars, mainly glucose and fructose, into ethanol and CO_2. This process is carried out by yeast such as *Saccharomyces cerevisiae* and *S. bayanus* [4], as well as by some bacteria, including *Zymomonas mobilis*, used in Central America in the fermentation of *Agave* to produce *pulque* [5] or palm wine (Toddy) [6]. The pyruvate is decarboxylated before a final reduction by NADH, to yield ethanol. The recovery of NAD maintains the flux of glycolysis reactions [7].

In addition, other by-products of fermentation are generated, such as glycerol, acetate, succinate, higher alcohols, and esters. The production of glycerol can be considered beneficial in some cases, that is, wine production, but is undesirable in the production of distilled beverage since it represents a waste of substrate [8]. Likewise, succinate production by yeast can have an important beneficial effect on the quality of *sake*, while it produces a negative effect on wine favoring a salty and bitter taste [9]. Esters represent an important group of flavor-active compounds with beneficial fruity/floral flavors and aromas in fermented beverages [7].

It should be noted that alcoholic fermentation could occur in aerobic environments. For example, even in the presence of abundant oxygen, yeast cells greatly prefer fermentation to oxidative phosphorylation, as long as sugars are readily available for consumption, a phenomenon known as the Crabtree effect [10].

2.2 Lactic and malolactic fermentation

Lactic acid fermentation is mainly a bacterial process that plays important roles in fermented beverages, enhancing its nutritional value and organoleptic quality. A group of morphologically and physiologically diverse bacteria has been designated the term lactic acid bacteria (LAB), due to the main production of lactic acid generated from the catabolism of carbohydrates [11]. They can be divided into two physiological groups, homo- and heterofermentative, depending on the hexose metabolic pathways used. Homofermentative LAB (*Lactobacillus delbrueckii* and

Streptococcus thermophilus) ferment hexoses via glycolysis (the Embden-Meyerhof pathway), producing lactic acid as the major end product, whereas the heterofermentative LAB (*Oenococcus oeni, Lactobacillus brevis, Lactobacillus hilgardii*, and *Lactobacillus buchneri*) and facultative homofermentative bacteria *(Lactobacillus plantarum*), in contrast, ferment hexoses and pentoses via the pentose phosphate or phosphoketolase pathway to produce acid lactic, CO_2, and ethanol and/or acetic acid [12].

Malolactic fermentation, the second important stage in winemaking, normally takes place after alcoholic fermentation. The malolactic fermentation is also conducted by LAB, preferably *Oenococcus oeni*, which reduce acidity of the wine or cider by transforming malic acid (dicarboxylic acid) to lactic acid (monocarboxylic acid) resulting in a softer taste [7]. In addition, the malolactic fermentation also affects the final aroma and taste balance by modifying and producing aroma-active compounds [12].

2.3 Acetic fermentation

Acetic fermentation, also called oxidative fermentation, is a process in which alcohol is oxidized to acetic acid by the action of a group conveniently called acetic acid bacteria (AAB). These are strict aerobic bacteria found in high-sugar, alcoholic and acidic environments, characteristics found in fermented beverage processes [13]. The AAB partially oxidate carbohydrates to generate aldehydes, ketones, and organic acids in the fermentative media [14]. AAB are evidently involved in the production of vinegar and participate in fermentation of other beverages, such as palm wine, pulque, and kombucha [15]. However, the main concern with this type of microorganisms is that they are involved in the spoilage of wine, cider, and beer, where the production of acid acetic is undesired [16].

2.4 Secondary metabolism

The metabolism of microorganisms is not a straightforward pathway, and other compounds are produced in lower concentrations during the metabolization of substrates, the so-called secondary metabolites.

Higher alcohols, polyols, esters, organic acids, vicinal ketones, and aldehydes are the main secondary metabolites produced in lower concentrations, as low as ng/L, althougth human senses are able to detect them due to the low perception threshold of these compounds, providing flavor and aroma to the fermented beverages [7].

Superior alcohols, also called fusel oils, are generated as by-products of the catabolism of amino acids, specifically by transamination reaction, which yields α-keto acid that enters the Ehrlich pathway, resulting in decarboxylation forming an aldehyde, and it is then oxidized to generate an alcohol [17]. Also, the aldehyde could be released or reduced to generate an acid.

Glycerol, the most important polyol, is formed during fermentation, as one molecule of glucose at some point is divided in two molecules of three carbons, one yielding glycerol and the other pyruvate [18].

Esters are formed by the reaction of an alcohol group and an acid group. The most important are the acetate esters, in which the acid group is originated from acetic acid and ethyl esters, where the alcohol group is from ethanol. Yeast produce esters to achieve the transport from cytosol to the fermenting medium as they are able to passively diffuse the cellular membrane [19].

Vicinal diketones are formed as intermediates of the biosynthesis of branched amino acids valine, leucine, and isoleucine [20].

2.5 Microbial stress and adaptation process during fermentation

During the fermentation process, yeast and LAB must respond to several adverse conditions, mainly low pH, increasing ethanol concentration, nutrient limitations, fluctuations of oxygen concentration, and the presence of diverse compounds with antimicrobial effects [21, 22]. One of the major stress response pathways is the global stress response, including the expression of heat shock factors [23]; this is activated by several environmental conditions, as a general non-specific cell response to adverse conditions. Likewise, specific adaptation strategies are triggered under certain circumstances. Adaptation of *S. cerevisiae* environmental conditions involves the activation and repression of different sets of genes during fermentation. For example, macromolecules transport and glucose signaling are repressed at initial stages of fermentation in synthetic must, while vacuolar activity is important as far as the beginning of stationary phase [24].

Yeast viability in stationary phase is fundamental to an efficient fermentation, some reactive oxygen species (ROS) could be produced and cause oxidative damage on lipids, proteins, and nucleic acids, including mitochondrial DNA. Cells respond with the production of proteins like superoxide dismutase and rhodanases [25]. Cellular accumulation of trehalose has been associated with increased resistance to oxidative stress and survival to low temperatures [22].

Assimilable nitrogen in must have a great influence over fermentation rate in wine—low nitrogen concentration leads to a low biomass yield and slow fermentation rate [26]. During nitrogen depletion different pathways are activated such as ammonium permease, nitrogen catabolic genes, post diauxic shift elements, and autophagy; all depending of target of rapamycin signaling [27].

LAB are recognized by their high acid tolerance, and indeed, malolactic fermentation is an adaptation response to reduce wine acidity, improving its survival [28]. Other strategies to respond to high acidity are citrate fermentation, amino acid degradation to produce alkaline substances, active proton pump, accumulation of trehalose and glutathione, and degradation of phenolic acids [12].

3. Strategies to improve desirable characteristics

In the past, the main objective for the selection of microorganisms was that they achieve fermentation in a relatively short time, with high conversions from substrates to the metabolites of interest and without the generation of compounds detrimental to the quality of the fermented food [29]. Nowadays, the characteristics sought for in fermentation processes have increased to satisfy the needs of more customers and producers which aim to increase flavor and aroma rather than ethanol concentration [30]. The focus on the use of a single strain to perform such deeds is considered impossible. This is why two main strategies have been proposed and evaluated, the use of multiple microorganisms each carrying out a specific function and as a whole produce the desired change, or the use of single microorganisms genetically modified to perform several tasks by themselves.

4. Microbial consortium

During beverage fermentations, two or more microbial groups living symbiotically define a consortium [31]. In food fermentation consortia, many aspects that are summarized as follows need to be considered: (1) different strains fulfill different and complex tasks, dividing work; (2) an adequate dynamic of the interactions

between microorganisms leads to stronger adaptability and stability of the consortium; (3) the participation of different microorganisms increases complexity in microbial dynamism, metabolism, transcriptomics, and interactions, that ultimately affect organoleptic characteristics of the product. Thus, along with the evolution of the medium, these microorganisms will establish relationships that will modify their individual behavior, determining temporal dominances, proportion of the participants, and thus major metabolites, which according to the substrate, will give organoleptically complex, microbiologically stable, and healthy products that consumers desire.

4.1 Main microorganisms present in some fermented beverages and their roles

It is still unclear how much mankind has intervened in the evolution of certain groups of microorganisms in fermented foods; however, it is clear that each substrate itself exerts a different selection pressure on them. In order to determine the diversity and evolution of a microbial consortium in any type of substrates, two approaches are available nowadays. First, the traditional microbiological methods, defined as culture-dependent, which may be biased by selectivity of culture media, low populations, and the presence of viable but non-culturable cells; however, it allows to further study individual behavior of isolates. The second approach is the culture-independent or molecular methods, which nevertheless may be affected by the specificity of primers, conditions of the reaction, detection of death cells, and database availability. Culture-independent methods have allowed to obtain a more complete scene, and combining with selective flow cytometry, metabolomics, and transcriptomic studies, a further comprehensive vision of microbial biodiversity of fermented foods can be reached [32]. Some of the most important fermented beverages are presented in **Table 1**, according to the type of dominant microorganisms and the raw materials used for their preparation.

Main microorganism	Raw material (substrate)	Examples	References
Saccharomyces yeasts	Fruit	Fermented teas, wine, cider, perry, fruit-fermented beverages.	[33–35]
	Dairy	Kumis, kefir	[36]
	Grains	Beer and distillates	[37]
Non-*Saccharomyces* yeasts	Fruits	Pulque and mezcal	[5]
	Dairies	Kumis, kefir	[36]
	Grains	African fura, Mexican pozol, South American champú, Asian rice wine, among others.	[38–41]
Lactic acid bacteria	Fruits	Pulque, Taberna, tomato juice, pomegranate juice	[5, 42–44]
	Dairies	Yogurt, kefir	[45, 46]
	Grains	Sourdough, Cocoa beans, Lambic beer	[47]
Acetic acid bacteria	Fruits	Kombucha, Water kefir	[33, 48]
Molds	Grains	Sake and soy sauce	[49]

Table 1.
Classification of some of the most common fermented foods produced worldwide according to the main groups of microorganisms and the starting substrate.

4.2 Interaction between microorganisms in mixed cultures

In order to survive in an environment, a group of different type of microorganisms need to adapt and specialize through the time they spend in it. Microbial relationships are needed to establish and maintain the microbial consortium; the type of interaction that can emerge may be positive as mutualism or synergism, in which both parts benefit from being together. However, the relationships can also be negative or antagonistic, when one microorganism inhibits another, for instance by nutrients or space competition; or by producing a metabolite that harms the other; or by presenting parasitism, in which one microorganism benefits at the expense of other, damaging and even killing it [50, 51]. Any type of interaction starts by recognizing the environment, then transferring the information to others. The phenomenon is regulated by mechanisms such as quorum sensing, which consists in a stimuli-response system that regulates gene expression in response to population density [51].

In the particular environment of beverage fermentations, as exhaustively reviewed by [50], microorganisms manifest a variety of interactions. During fermentation, the environment generated maintains most of human pathogenic or food spoilage microorganisms. This role is achieved through competition and antagonism, through the fast consumption of nutrients and production of inhibitory compounds, mainly ethanol and organic acids, usually acting together with medium, short-chain fatty acids and proteinaceous toxins such as yeast's killer toxin in wine. On the other hand, throughout the evolution of the original substrate, the limiting factors change and the dominant microorganisms also change along with them. This succession of species has been reported in almost every fermented food studied. Positive interactions determine largely the succession of microbes in a particular substrate, for instance in *sake* production, where the saccharification of starch by *Aspergillus flavus* var. *oryzae* is first required in order to let *S. cerevisiae* conduct the alcoholic fermentation [52].

Besides the simply descriptive craving to know the diversity and roles that each microorganism plays, by understanding the types of interactions and how they emerge, a more controllable process can be achieved and the quality of the products can be improved. Finding the combination of microorganisms (species and strains) that will give desired characteristics is a strategy vastly explored in wine [53, 54], and also in *cachaça* [55], prickly pear wine [56] where mixed populations of *Saccharomyces*, non-*Saccharomyces* yeast, and even LAB have been explored.

One important aspect to consider when a proper combination of microorganisms is sought is to investigate their compatibility, that is, not negative type of interaction, as well as to determine if the intended promoting role actually occurs during the fermentation process. For instance, regarding compatibility, a study was conducted to observe synergism, antagonism, or no apparent interaction between selected native yeasts and LAB strains for the production of wine in the region of Queretaro, Mexico [57]. For this, yeast strains were grown in a medium resembling must, after 12 h yeast biomass was removed and the resulting broth was used to incubate the different strains of LAB and to observe their growth by means of optical density (OD) (**Figure 1**).

Positive values indicate a growth promotion from yeast to LAB observed in different extent, showing synergism superior in the combinations of native yeast strains compared with the growth promotion given by the commercial yeast (K1-V1116). It is also observable that the behaviors were strain-combination dependent, an aspect cited by other authors [58]. This test allowed to foresee and select compatible strains in order to further analyze their performance in a traditional winemaking process, where LAB strain is inoculated after the alcoholic fermentation performed by the yeast strain.

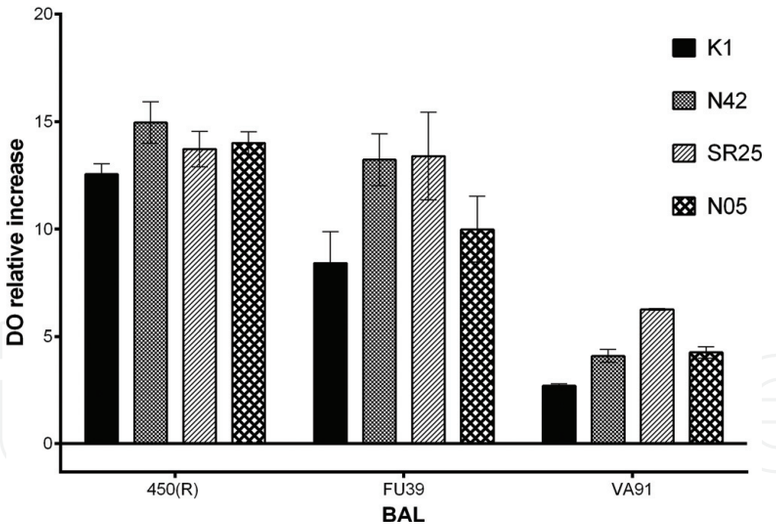

Figure 1.
Compatibility of four native LAB grown into the medium produced by five native S. cerevisiae *strains (N42, SR25, N05), measured as relative optical density increase* $\left[\mathrm{ODi} = \frac{\mathrm{ODi}_{\mathrm{afteryeast}} - \mathrm{ODi}_{\mathrm{withoutyeast}}}{\mathrm{DOi}_{\mathrm{withoutyeast}}}\right]$. *Strain 450®* (O. oeni) *and K1-V1116® (K1) were used as commercial references.*

In a different context, regarding a particular metabolic interest or synergism, a study carried out in tequila fermentation is briefly presented. An important safety issue in the consumption of tequila (and in general, distillates) to take into account is the elevated concentration of ethyl carbamate generated by the reaction between urea and ethanol driven by the elevated temperatures occurring during the distillation stage. While ethanol is the desired metabolite in this process, urea is the by-product of nitrogen metabolism of *S. cerevisiae* and thus its production cannot be totally eliminated. On the other side, bacteria are capable of consuming urea as nitrogen source [59]. Taking advantage of the usual symbiosis across yeast, LAB, and AAB, an alternative approach that has been explored to reduce ethyl carbamate production is the use of mixed cultures, combining a selected *S. cerevisiae* strain and bacteria strains isolated from spontaneously fermenting agave juice (**Figure 2**).

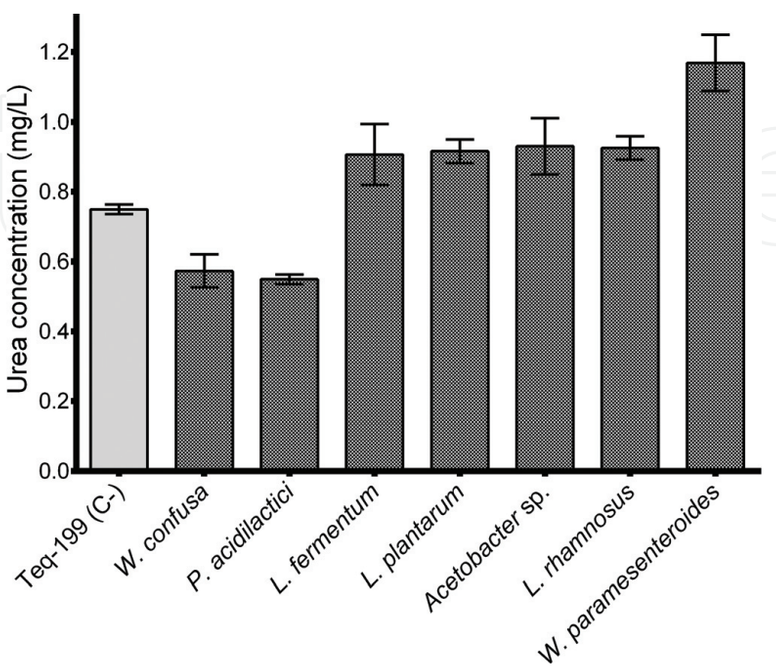

Figure 2.
Urea concentration produced by S. cerevisiae *strain Teq-199 individually (C-) or in combination with seven native bacteria species (data not published).*

Compared with fermentation individually carried out by *S. cerevisiae* strain, a clear tendency to decrease urea concentration of approximately 0.2 mg/L was observed when *Weissella confusa* and *Pediococcus acidilactici* were co-inoculated with the *S. cerevisiae* strain. Conversely, a moderate increase was obtained with the rest of the bacterial strain, especially with *Weissella paramesenteriodes*, with an increase of about 0.4 mg/L compared with the control. These changes are respectively associated with a consumption and production of the metabolite in question, depending on the species used.

These cases exemplify some of the strategies that have been followed in order to choose or validate the use of mixed cultures, seeking to achieve particular objectives and trying to ensure the success of combining certain strains.

5. Genetically modified microorganisms

Natural genetic differences are shown in strains of the same species. This variability can be replicated under laboratory conditions intended to improve characteristics of microorganisms [60]. These traits could be modified by directed or by "natural" methodologies. Even though both approaches result in genetically modified microorganisms (GMMs), the laws that dictate the feasibility on food production depend on the strategy used [61].

It is necessary to consider that the strains to be modified for food fermentation must be labeled as generally recognized as safe (GRAS) or qualified presumption of safety (QPS), not related with pathogens; so, they should be taxonomically identified, as well as being genetically stable under industrial processes [62]. Under these considerations the most investigated eukaryotic microorganism is *S. cerevisiae*, used for several centuries for food and alcoholic production; thus, their metabolic pathways and gene-related regulation are well known. Furthermore, the genome of this species has been completely sequenced, providing the basis for applications of genetic engineering [63]. Meanwhile, technological improvement investigation has been carried out mainly on LAB (**Table 2**).

5.1 Directed genetic modifications

The directed modification is carried out by genetic engineering causing a punctual manipulation in a known region in the genome that in turn will improve a characteristic of interest or the repression of a negative trait. The changes usually involve the promoter region to induce or repress gene translation, or the deletion or insertion of new genes from other microorganisms. This approach presents several drawbacks in food industry. First, it requires the global knowledge of metabolic pathways, genes involved, and their regulation [79]. Second, a single gene modification cannot produce the expected result, since some pathways are regulated by several genes, making a complex process to obtain the desirable trait [61]. And third, the use of microorganisms modified this way is prohibited in foods by law in the European Union, USA, and other countries [80].

The only permitted directed genetically engineered strain used in USA is a *S. cerevisiae* strain able to fully carry out a malo-alcoholic fermentation. This strain was generated by the integration of a malate permease gene from *Schizosaccharomyces pombe* and malic enzyme from *O. oeni* to the constitutive promoter of the 3-phosphoglycerate kinase of *S. cerevisiae* [81].

Modification technique	Species	Modified trait	Reference
Adaptive evolution	*S. cerevisiae*	Flocculation in the surface	[64]
	S. cerevisiae	Ethanol reduction and flavor increase	[65]
	S. cerevisiae	Ethanol reduction	[66]
Random mutagenesis	*L. lactis*	Domestication from plant to milk fermentation	[67]
	Yeast (species not identified)	Reduction of acetic acid	[68]
	O. oeni	Malolactic efficiency and sensory properties	[69]
Natural conjugation	*S. cerevisiae* *S. bayanus*	Fermentation at low temperature	[70]
	S. cerevisiae *S. bayanus*	Stress resistance and fermentation performance	[71]
	S. cerevisiae *S. paradoxus* *S. pastorianus*	Aroma production	[60]
	S. cerevisiae	Determine gene implicated in nitrogen requirements	[72]
	S. cerevisiae	Acid- and thermo-tolerance	[73]
Genome shuffling	*S. cerevisiae*	Improve fermentation performance, affected negatively the flocculation capacity	[74]
	S. cerevisiae	Improve fermentation performance	[75]
	S. cerevisiae	Improve fermentation performance	[76]
	Candida krusei	Improve acetic acid tolerance	[77]
	Acetic acid bacterium	Improve tolerance of ethanol	[78]

Table 2.
Examples of genetic modifications applied to microorganisms for fermented beverages improvement.

5.2 Natural genetic modifications

To obtain microorganisms with desired genetic characteristics using natural techniques, growth conditions are guided in the laboratory to improve the probability of inducing the desired genome modifications. All these natural techniques target the whole genome of the strain, generating several different genotypic changes and, thus, generating the need to further select the strains with the phenotypic variation desired. These methodologies are "allowed," or at least not prohibited by the law as they do not enter in the legal definition of GMM [30]. Among other strategies, some of the most important are described below.

5.2.1 Adaptive evolution

In this methodology, strains are grown in a medium exerting an increasing selective pressure to allow the most adapted generations to become dominant. During the replication of DNA, mutations could accumulate in the offspring without causing an evident modification. However, in a selective condition, only strains with the genetic pool needed to maintain the homeostasis of the cell under the stress pressure will be able to grow [82].

Adaptive evolution has been applied to divert ethanol to glycerol production, then reducing ethanol graduation in wine. It was achieved by increasing osmotic stress with salts in growth media. Glycerol is produced and accumulated in the interior of the yeast cell to counteract the osmotic pressure in the environment [66].

5.2.2 Random mutagenesis

The exposure of microorganisms to physical factors such as UV light, or chemical mutagens as alkylating agent, allows increasing the rate of mistakes in the replication. The offspring then are screened to select colonies with improved characteristics. The randomness of the mutations causes a big drawback, and the modification of regions other than the target of interest could impact negatively on the performance [83]. Also, as the genes occur in more than one copy in the genome, the mutation should be present in all the copies to obtain a strain with changed phenotype [84].

5.2.3 Natural conjugation

This methodology mainly has been applied to yeast, in which two strains, both having an interesting characteristic are crossed using their sexual cycle, thus also receiving the name of direct mating [60]. The resulting hybrid strain contains half genes from each parental strain, meaning that it will obtain some characteristics and lose others [85]. To discriminate the new hybrids from the parental strains, the latter must be differentiated, usually using respiratory-deficient and auxotrophic strains, which in turn only hybrids with prototrophy and respiratory proficiency would be able to grow in a selective media [60].

The most famous yeast strain generated by natural hybridization is the lager beer *S. pastorius*, having characteristics of *S. cerevisiae* and cryotolerance of *S. eubayanus*, which gave the desired fermentative proficiency at low temperatures [37]. Laboratory hybridization of *S. cerevisiae* x *S. mikatae* has also generated strains with improved and diverse volatile compounds that provide complexity to wines [86]. In addition, a hybrid of *S. cerevisiae* and *S. kudriavzevii* accumulated more glycerol, providing more cryotolerance, osmotolerance, and ethanol tolerance [87].

The major drawback of the sexual reproduction in yeast is that industrial strains poorly sporulate [61]. Rare mating is applied in these cases, switching the mating type of diploid or polyploid cells, and then being able to hybridize with the contrary mating type, to generate a new hybrid [88].

5.2.4 Cell fusion

In this methodology, the cell wall is disrupted generating spheroplasts that will spontaneously fuse to other cells, integrating their DNA into a single cell and, then, recombination occurs. The insertion of genetic material could be done even from microorganisms of other kingdoms [89].

Genome shuffling is based on protoplast fusion and nowadays several methodologies are integrated to provide complex phenotypes. It involves the induction of mutagenesis in a population of a specific strain, and then this new genetically diverse population could be screened by the evaluation of individual isolates or by applying a selective pressure to the media containing the mutants. The resulting exceptional mutants are hybridized by protoplast fusion or by mating. The resulting combinations could be further hybridized repeatedly to improve characteristics of

interest [75]. As this methodology is relatively new, their evaluation at industrial level to provide certainty of the results is still needed.

5.2.5 Horizontal gene transfer

In nature, horizontal gene transfer occurs in fungi and bacteria kingdoms, it involves the insertion of sequence elements, conjugation, transformation, and transduction from one microorganism to another [90]. These transferences could happen in non-taxonomically related microorganisms. In yeast, this mechanism is not well known; however, it has provided important features such as the identified in a *S. cerevisiae* strain by whole genome sequencing, in which a total of 34 genes were found to be transferred from non-*Saccharomyces* and *Zygosaccharomyces bailii*, providing important fructose fermentation capability [30].

Regarding bacteria, mating process involves close physical contact between a strain that donates its genetic material, mainly a plasmid, to a recipient. The vast majority of the plasmids transferred do not contain any technological use [91]. In LAB, important plasmids naturally present provide the ability to ferment lactose, gain resistance to bacteriophages, and produce bacteriocins [92]. Plasmids could also encode for antibiotic resistance and further transferring could occur to other species of importance to pathogenic bacteria [93].

6. Trends and perspectives

During the last years, there has been an increase in the demand of natural, artisanal, and organic-labeled products, leading to a rise in the request for autochthonous starters, which reflect the biodiversity of a particular area, supported by the idea of microbial "terroir."

An alternative to the use of single-strain starter cultures, which leads to very standardized products, is the use of autochthonous mixed starters (consortia), able to mimic the natural biodiversity, increasing organoleptic properties, but still maintaining controllable processes [52, 53].

On the other hand, considering the fact that mixed populations can perform functions that are difficult or even impossible for individual strains or species to do, nowadays the theoretical support to successfully obtain synthetic microbial consortium exists and presents a wider application potential than single synthetic cells. Taking into consideration the knowledge acquired on naturally occurring microbial interactions, the application of such technology seems feasible and attractive for many industries. This approach would make it possible to efficiently complete many tasks and to acquire a specific product profile compartmentalizing molecular components of each pathway, transcriptional regulators, and chemical intermediates in each different microbial individual. Nevertheless, the use of this technology would face many drawbacks until it is approved to be used in fermented foods, in spite of being the focus of several studies in other similar fields [94-96].

7. Conclusions

The genetic modification of strains and the development of mixed starter cultures aim for similar objectives, to improve the characteristics of fermented beverages maintaining control of the process and quality of the products. Both approaches possess strengths and weaknesses. While some advocate that changes in the genome open a vast opportunity to achieve all the desired characteristics in

fermented beverages, the other groups remark that only natural diversity and traditional methods could generate best products with typicity. Furthermore, the application of genetic modifications is badly perceived by consumers and legally prohibited in some cases. It seems that the next step in the improvement agenda is the combination of both approaches, the incorporation of mixtures of natural, genetically modified microorganisms and native strains to provide a holistic solution to the existing difficulties in fermentation.

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Conflict of interest

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