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Contribution of the Microbiome as a Tool for Estimating Wine's Fermentation Output and Authentication

*Dimitrios A. Anagnostopoulos, Eleni Kamilari
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Abstract

Wine is the alcoholic beverage which is the product of alcoholic fermentation, usually, of fresh grape must. Grape microbiome is the source of a vastly diverse pool of filamentous fungi, yeast, and bacteria, the combination of which plays a crucial role for the quality of the final product of any grape must fermentation. In recent times, the significance of this pool of microorganisms has been acknowledged by several studies analyzing the microbial ecology of grape berries of different geographical origins, cultural practices, grape varieties, and climatic conditions. Furthermore, the microbial evolution of must during fermentation process has been overstudied. The combination of the microbial evolution along with metabolic and sensorial characterizations of the produced wines could lead to the suggestion of the microbial terroir. These aspects are today leading to open a new horizon for products such as wines, especially in the case of PDO-PGI products. The aims of this review is to describe (a) how the microbiome communities are dynamically differentiated during the process of fermentation from grape to ready-to-drink wine, in order to finalize each wine's unique sensorial characteristics, and (b) whether the microbiome could be used as a fingerprinting tool for geographical indication, based on high-throughput sequencing (HTS) technologies. Nowadays, it has been strongly indicated that microbiome analysis of grapes and fermenting musts using next-generation sequencing (NGS) could open a new horizon for wine, in the case of protected designation of origin (PDO) and protected geographical indication (PGI) determination.

Keywords: grape, wine, microbiome, terroir, fermentation, next-generation sequencing

1. Introduction

Fermented products are generated as a result of metabolic activities conducted by functional microbes, leading to the biochemical and organoleptic modification of the substrates in order to meet the requirements of the consumers [1]. The dynamic interaction between the members of the microbial communities guiding

the process of fermentation has great influence in the nutritional, hygienic, safety, and organoleptic characteristics of the final product [2]. In a large number of fermented products, the formation of microbial biodiversity existing in the initial substrate is affected by a large number of factors, including the geographic origin, the cultural practices, differences among varieties, or the climatic conditions [3]. The contribution of the microbial community configuration, which is governed by spatial factors, land topography, environmental factors, etc. that sustain the spatial structure of the inhabitants, and their potential relation with the metabolic and sensorial characterizations of the final product, has been under deep research, leading to the suggestion of the microbial terroir [4]. The perspective of analyzing the microbial communities' dynamics as progressively differentiated during the process of fermentation for the determination of microbial terroir has been applied in grapes and consequently its final fermented product, the wine [5, 6].

Traditionally winemaking process relies on spontaneous fermentation, which is conducted without the addition of chemical compounds or supplementary microbes at the beginning of the fermentation process. Under spontaneous fermentation conditions, the microbial community participating in fermentation and which is responsible for the quality of the final product is considered to be quite unpredictable. At the initial stages of fermentation, the microbial communities are comprised by a rich biodiversity of several yeast and mold species, including *Metschnikowia*, *Candida*, *Hanseniaspora*, *Pichia*, *Lachancea*, *Kluyveromyces*, and *Saccharomyces* [7–9]. During must fermentation, the alcoholic fermentation conducted elevates the ethanol content and establishes the basic fermenters, such as *Saccharomyces cerevisiae*, among the predominant species [9]. Their dominant presence during the fermentation process has led to the isolation of several *S. cerevisiae* strains, which have been extensively studied for the potential application of their technological characteristics [9–11].

The development of high-throughput sequencing technologies has allowed the evaluation of the microbial consortium comprising grapes' microbiome in terms of revealing the concept of the microbial terroir [12–16]. The contribution of origin-associated factors of grape varieties, including climate and microclimate, region site, as well as grape cultivar, in the microbial community formation and the final metabolic profiles, has been recently investigated [12, 17–20]. These studies have led to an improved spatial and temporal determination of the wine grapes' microbiome and brought new insights into its dynamics and biodiversity, revealing a new horizon for the better characterization of this product, especially in the case of PDO and PGI wines' designation. These labels were established by the European Union (EU) to guarantee the authentication of the local products produced in distinct geographic origin, applying traditional specialties. Metagenomic studies have been recently applied to identify the microbial communities that influence the original sensorial characteristics of PDO wines [14, 16].

The aim of this chapter is to extensively review all latest literature in the scope to investigate (a) how the microbiome communities are dynamically differentiated from grape to ready-to-drink wine, in order to finalize each wine's unique sensorial characteristics, and (b) whether the microbiome could be used as a fingerprint tool for regional characterization, based on high-throughput sequencing (HTS) technologies.

2. Methods to identify grape microbial species

Grapes are comprised by a complex microbiome, the members of which share different physiological characteristics and effects upon wine production. Some of them are present only in grapes and soil, such as parasitic fungi and environmental

bacteria, while others have the ability to survive and grow during wine fermentation, constituting the wine microbial consortium. Several studies over the last years have reported that the biodiversity and the quantity of the microorganisms present on the surface of the grape berry are highly dependent on many factors, including the health state of the grapes, the temperature, the microclimate conditions, and the pesticide treatments [21–23]. Recently, the “terroir” idea was proposed to be extended to the microbiological aspect, indicating that the geographical distribution of the grape and soil microbiota is not randomly dispersed but is dependent on the cultivar, the location of the vineyard, and the vintage [17].

The application of culture-dependent methods is considered weak to support the terroir perspective, since less than 1% of the total population can be detected [24], and these methods also fail to detect viable but non-culturable organisms [25–27]. Additionally, the stressful environment shaped during winemaking due to the addition of SO₂, high ethanol concentration, etc. forces a number of bacteria and yeast to enter a viable but non-culturable state (VBNC) [28, 29]. Even though still viable and maintaining a detectable metabolic activity, the microbial cells are unable to grow on culture media during VBNC status [30]. Examples of such microorganisms include *Candida stellata*, *Brettanomyces bruxellensis*, *S. cerevisiae*, and *Zygosaccharomyces bailii* [27]. In order to study the existence of bacteria during VBNC, microbiologists have applied alternative culture-independent techniques. Three of the main culture-independent techniques applied include quantitative real-time PCR (qPCR), restriction fragment length polymorphism (RFLP), and denaturing gradient gel electrophoresis (DGGE) [24, 27, 31–33]. Still, the detection sensitivities of these techniques remain limited due to the predominance of certain yeast such as *C. zemplinina* and *S. cerevisiae* during fermentation, which restrict the detection of low-abundant species.

The introduction of next-generation sequencing (NGS) technologies has significantly enhanced the information elicited from microbiological studies, allowing the distinction of the high-abundant species from the low-abundant, with detection sensitivities greatly higher than the previously used molecular techniques [24]. For instance, analysis of the microbial communities' formation existing on grape and during Carignan and Grenache must fermentation from three vineyards in Priorat (Spain) highlighted the ability of NGS to detect an increased amount of species compared to DGGE [34]. Undoubtedly, NGS provides a new powerful tool, with elevated capabilities to enhance the understanding of the complexities of microbial communities as dynamically differentiated from grapes and its close environment to ready-to-drink fermented wine, in terms of diagnostic, monitoring, and traceability [16, 21, 35–38]. Understanding the progressive alterations of the microbial diversity during fermentation using HTS technologies is considered a promising approach to reveal correlations between microbiomes and geographical origin.

3. Identification of the microbial communities

Terroir is characterized by a multi-complex ecosystem where the vine (genetic material and cultural practices) interacts with the environmental factors (i.e., soil, climate, microclimate, humans, etc.) affecting the quality and typicity of the wine produced in a particular location. The understanding of the microbial terroir involves the identification of the microbes shaping grapes' environmental communities and the evaluation of their diversity dynamical evolution throughout the different stages of fermentation, until wine production. During natural fermentation the complex microbial communities that comprise the grape microbiome, including, yeasts, yeast-like fungi, and bacteria, are under the selective pressure of the

alterations in the must microenvironment, caused by microbial interactions, as well as chemical and physical factors [39]. The must microbes have to handle stressful factors that affect their survival, including reduced oxygen, high ethanol and sulfur dioxide (SO₂) levels, and low pH [40]. Moreover, the amounts of sugar existing in must favor for particular species, and high sugar content sweet wines select for osmotolerant species [41, 42]. As a consequence of this stressful microenvironment, numerous environmental species become unable to survive, while others, which are able to perform alcoholic fermentation and were detected in reduced relative abundance before fermentation, such as *Saccharomyces cerevisiae*, become dominant by the end of fermentation [16]. Apart from alcoholic fermentation, malolactic fermentation (MLF) (conversion of malic acid into lactic acid) is also involved in the metabolic transformation of grape juice into wine, conducted mostly by lactic acid bacteria (LAB), including the genera *Oenococcus*, *Lactobacillus*, *Pediococcus*, and *Leuconostoc*, leading to must deacidification, a process that affects organoleptic characteristics' formation [43]. By the end of fermentation, the microbial diversity is limited to selected microbial species [12, 35]. As revealed by several studies, some species were found to decline rapidly at the initial or the middle stages of fermentation, such as *Cryptococcus carnescens*, *Paraburkholderia terricola*, *Aureobasidium pullulans*, and *Metschnikowia pulcherrima*, while others exist until the end of fermentation, including *Saccharomyces cerevisiae*, *Torulaspora delbrueckii*, *Lachancea thermotolerans*, and *Streptomyces bacillaris* [44–47].

Overall, the fungal population at a phylum level is very similar and mainly comprised by *Ascomycota*, the most abundant phylum, followed by *Basidiomycota* [3, 18, 19, 24, 35, 48]. Additional phyla frequently detected but in limited concentrations include *Zygomycota* and *Chytridiomycota*. The most commonly found filamentous fungi genera include *Aspergillus*, *Erysiphe*, *Alternaria*, *Cladosporium*, *Penicillium*, *Davidiella*, *Lewia*, *Botrytis*, as well as the yeast-like fungus *Aureobasidium pullulans*. Further yeast genera commonly found include *Issatchenkia*, *Candida*, *Hanseniaspora*, *Pichia*, *Rhodotorula*, *Metschnikowia*, *Lachancea*, *Filobasidiella*, *Cryptococcus*, *Torulaspora*, and *Sporobolomyces* [3, 18, 19, 24, 34, 35, 48, 49].

High-throughput sequencing studies have been applied to evaluate the bacterial communities associated with the vineyard. The most frequently detected phyla in vineyard soils and grapevine roots include *Proteobacteria*, *Bacteroidetes*, *Acidobacteria*, *Verrucomicrobia*, *Planctomycetes*, *Actinobacteria*, *Chloroflexi*, *Gemmatimonatedes*, and *Firmicutes* [21, 50–52]. High-throughput analysis of the grapevine phyllosphere, flowers, and grape berry surface indicated that the bacterial communities were predominated by *Proteobacteria* followed by *Firmicutes*, *Actinobacteria*, *Acidobacteria*, and *Bacteroidetes* [13, 38, 53, 54]. The relative abundances of the groups may vary depending on the plant tissue or organ. The dominant taxa include members of the genera *Pseudomonas*, *Sphingomonas*, *Frigoribacterium*, *Curtobacterium*, *Bacillus*, *Enterobacter*, *Acinetobacter*, *Erwinia*, *Citrobacter*, *Pantoea*, and *Methylobacterium* [3, 13, 21, 48, 53, 54]. In contrast, the endophytic community in grape berries is mainly comprised by *Ralstonia*, *Burkholderia*, *Pseudomonas*, *Staphylococcus*, *Mesorhizobium*, *Propionibacterium*, *Dyella*, and *Bacillus species* [35].

4. Factors affecting the microbial communities' formation

Grapevines' associated microbial communities originated from distinct geographic regions exhibit different profiles [13, 18, 34, 36, 55]. Each region is differentiated by the dominance of a few species per region. Indicatively, *Aspergillus* and *Penicillium* spp. were largely associated with the Chardonnay in Napa, while *Actinobacteria*,

Bacteroides, *Saccharomycetes*, and *Erysiphe necator* dominated in Central Coast, as well as *Proteobacteria* and *Botryotinia fuckeliana* in Sonoma [3]. Additionally, the prevalence of *Lachancea* in the Alentejo appellation was reported by Pinto et al. [13] while of *Rhodotorula* and *Botryotinia* was shown in the Estremadura appellation. Finally *Ramularia* and *Hanseniaspora* were the dominant genera in Bairrada, *Rhodotorula* and *Lachancea* in Dão, *Rhodotorula* and *Erysiphe* in Douro, and *Rhodotorula* and *Alternaria* in Minho appellation. Furthermore, the fungal grapes' associated diversity is also affected by agronomic practices. Vineyards that employed conventional, integrated pest management systems, organic, biodynamic, and ecophyto practices were shown to harbor different fungal communities [19, 23, 24, 44, 46, 48, 56–59]. However, the fact that these studies were carried out in vineyards from different countries (Austria, France, Italy, Spain, and Slovenia), subjected to different climates, pesticides, and regulatory constraints, may explain the contradictory results.

Many studies suggested that yeast diversity is dependent on climatic and micro-climatic conditions. Higher yeast diversity has been described for vintages with high rainfall [40, 57] probably due to substantial fungal proliferation. Dry wines are produced by grapes submitted to prolonged withering in order to become moderately dried. The climate, as well as the extent of the withering period, was found to affect the formation of the fungal microbiome on grape skins in *V. vinifera* L. cv. Corvina, influencing the relative abundances of the fungal genera and consequently the secreted metabolites shaped in the must of Amarone red dry wine [57]. Grapes collected during a rainy season had increased bacterial biodiversity and enriched volatile compound (VOC) profile compared to a “dry” season collection, although some common microbial populations and VOC profiles maintained over the different vintages in grapes and musts samples, probably indicative of the typicity of Amarone.

Vineyard factors such as grape variety and berry chemical components are often described to influence microbial diversity [11, 43, 61, 62]. For instance, in similar soil and climatic conditions, *Cryptococcus* was the genera most frequently isolated (90% of all isolates) from Grenache grapes, whereas *Hanseniaspora* was the genus most frequently isolated from Carignan (75%) [58].

The health status of berries can also affect the diversity of yeasts. The ascomycete *Botrytis cinerea* is considered one of the most damaging fungi in low temperature viticulture [60]. It causes *Botrytis* bunch rot, alternatively gray mold in grapes, affecting the physiochemical condition of grapes dramatically. Botrytized wine fermentations were found to contain increased abundance of acetic acid bacteria (AAB) in comparison with unaffected wines [61]. The elevated presence of AAB was additionally shown in botrytized wine fermentations obtained from the Dolce Winery, Oakville, California, analyzed via HTS [36]. Interestingly, the lactic acid bacteria (LAB) community was comprised mostly by *Leuconostoc* and *Lactococcus*, whereas *Oenococcus* was completely absent. Berries affected by *Botrytis cinerea* indicated increased development of the genus *Metschnikowia* [62]. Additionally, the bacterial community structure may vary depending on the grape cultivars or the agronomic practices [13, 35, 48, 52, 53].

One of the factors found to contribute to microbial communities' formation is the amount of SO₂. Comparison of the bacterial community dynamics following the fermentation process of hand-harvested organically grown Riesling grapes following organic and conventional *pie-de-cuve* (PDC) indicated that the species *Gluconobacter oxydans* was significantly affected by the addition of SO₂ prior to PDC and bulk fermentation [37]. The ability of SO₂ to prevent the growth of *Gluconobacter* at concentrations ≥25 mg/L was also shown by Bokulich and colleagues [63]. The elevated presence of this spoilage bacterium in organic fermentation highlights the susceptibility of the organic fermentation procedures to wine spoilage.

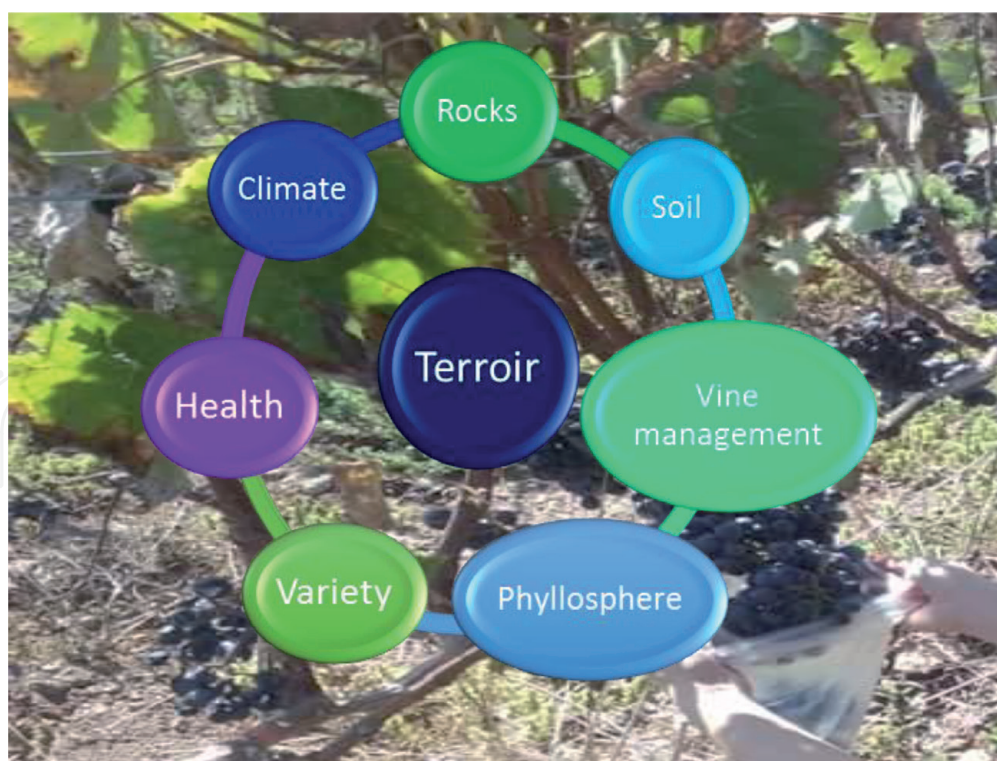


Figure 1.
Variables related to the microbiome formation.

Generally, many of these variables (e.g., climatic conditions or cultivar) are interdependent and may be clustered into broad groups of effects (**Figure 1**). The study of Bokulich and Mills [17] has shown that grape-associated microbial region is totally related with varietal, biogeographical, and climatic factors across multiscale viticultural zones. According to other study [20], the distribution of yeast species promotes significantly intra-vineyard spatial fluctuations. Continuously, the heterogeneity of grape samples harvested from single vineyards at the same stage of ripeness might be related, at least in part, to differing microbial communities in different sections of the vineyard. The biodiversity of yeast species in grapes is affected by numerous biotic and abiotic factors, as well as the interactions among the resident populations. However, more studies need to be performed in order to confidently elucidate the vineyard and grapevine phyllosphere microbiome.

5. Microbial evolution of must during spontaneous fermentation process

High-throughput sequencing techniques have allowed the discrimination of the microbial diversity as dynamically formed from the initiation of fermentation until wine production, identifying also the non-culturable microorganisms, as well as the limited represented species [12–16] (**Figure 2**). During the process of fermentation, the microbial community is reshaped and become dominated by the fermentative organisms. These alterations, however, are to a large extent dependent from the origin of the must/wine, including the winery and the grape variety [12]. Metagenomic analysis of the microbial communities' structure fluctuations formed throughout the fermentation of grapes obtained from American Viticultural Areas (AVA), for Cabernet and Chardonnay wines production, combined with metabolomic analysis, indicated that the characteristic microbial signatures of grapes and soil disappeared during fermentation to become replaced by characteristic fermentative microbes, but still, the microbial and wine metabolite profiles were able to distinguish the

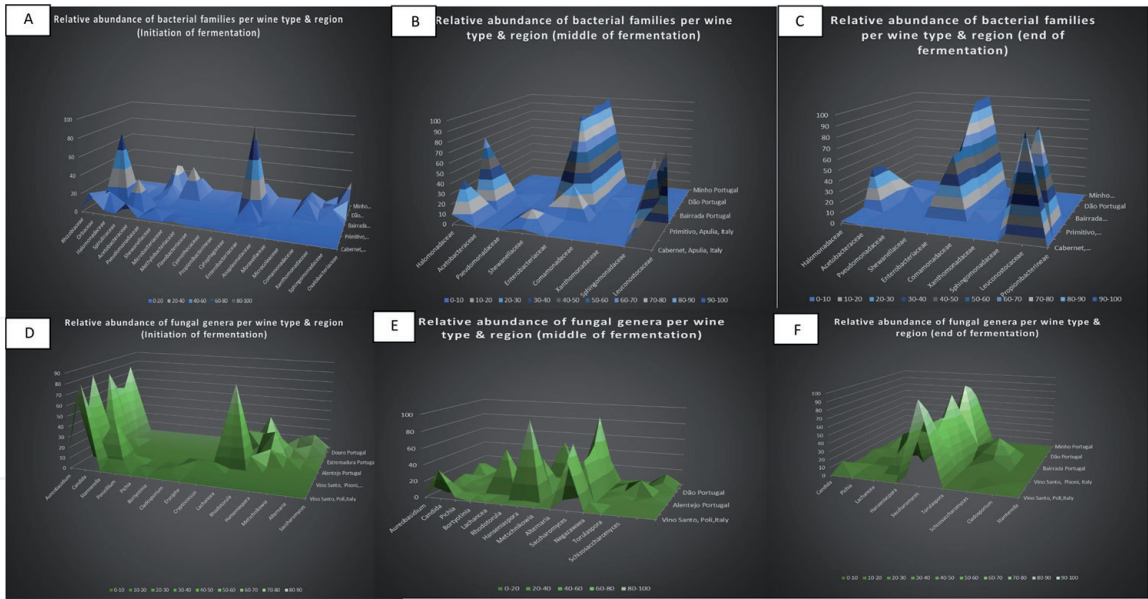


Figure 2.
Spatial distribution of the microbial communities shaping from the initiation of fermentation until wine production regarding the studies of Stefanini et al. [16], Marzano et al. [14], Wei et al. [15], and Pinto et al. [13]. (A) Representation of the relative abundance of bacterial families at the beginning of fermentation. (B) Representation of the relative abundance of bacterial families at the middle of fermentation. (C) Representation of the relative abundance of bacterial families at the end of fermentation. (D) Representation of the relative abundance of fungal genera at the beginning of fermentation. (E) Representation of the relative abundance of fungal genera at the middle of fermentation. (F) Representation of the relative abundance of fungal genera at the end of fermentation.

individual vineyards and the viticultural area, as revealed by random forest machine learning models [12]. Markedly, a negative association among the fermentation rate as well as bacterial richness with various taxa, such as *Lactobacillus* spp., *H. uvarum*, and *Gluconobacter*, was observed, indicative of the ability of some bacteria to prevent alcoholic fermentation, probably due to antagonism for available nutritional sources with the alcoholic fermentation fermenters, such as *S. cerevisiae*, while others, such as *Pseudomonas*, were positively correlated in both wines. The malolactic fermentation (MLF) conducted in Cabernet limits the bacterial biodiversity of wines to the presence of members of the family *Leuconostocaceae* (*Oenococcus oeni*), whereas the fungal biodiversity, as well as the microbial diversity of Chardonnay wines, remained enriched throughout fermentation and wine production, possibly responsible for the more distinct both regional and vineyard discriminations of Chardonnay wines compare to Cabernet Sauvignon wines.

In order to understand the association among the biogeographic distribution of wineries and wine microbiome of six different Portuguese wine appellations, HTS analysis was applied to reveal the dynamics of microbial communities' formation following the different stages of spontaneous wine fermentations [13]. The presence of an increased average microbial biodiversity dissimilarity among the grape microbiome from the different wine appellations (60.16 and 57.36% for eukaryotes and prokaryotes, respectively) indicated the elevated contribution of the vineyard environment in microbial communities' shaping and consequently the influence of the initial microbiome to the uniqueness of the different appellation-derived wines. During the process of fermentation, the average microbial dissimilarity was reduced, due to alterations in the microbial biodiversity and dominance of specific, able to perform fermentation species, leading to the loss of the biogeographic profile, but still each wine was distinguished by its unique pattern of microbial biodiversity.

The high detection sensitivities of HTS technologies have allowed the identification of the rich bacterial biodiversity implicated in Cabernet, Negroamaro, and

Primitivo Apulian red wines' production process, highlighting the alterations in the bacterial population during vinification [14]. Although a common microbiome core was identified among the three wine varieties, comprised by the genera *Candidatus liberibacter*, *Gilliamella*, *Gluconobacter*, *Halomonas*, *Halospirulina*, *Komagataeibacter*, *Pseudomonas*, and *Shewanella*, each wine was discriminated by a unique taxonomic signature. During malolactic fermentation *Shewanella*, *Halomonas*, and *Oenococcus* became the dominant genera, whereas at the end of fermentation, *Oenococcus*, with the species *Oenococcus oeni*, became the abundant bacterium of the three wines' microbiome. Similarly, HTS analysis of Cabernet Sauvignon samples from three different winery regions in Xinjiang province, China, from Fukang area, identified a common core microbiome composed mostly by the fungal genera *Aureobasidium*, *Pleosporaceae*, *Cryptococcus*, and *Dothideales* and the bacterial genera *Pseudomonas*, *Acinetobacter*, *Kaistobacter*, *Arthrobacter*, and *Sphingomonas* in all grape and grape juice samples analyzed, even though the relative abundances of those genera were different [15]. However, following malolactic fermentation, the microbial biodiversity was gradually reduced and limited mostly to the fungal genera *Aspergillus*, *Penicillium*, and *Alternaria*, while the slow-growing, necessary for malolactic fermentation, lactic acid bacterium *Oenococcus* appeared to be the dominant genus in all wine samples.

Metagenomic analysis, applied to reveal the spatial distribution of the microbial communities shaped in Vino Santo Trentino sweet wine, produced by Nosiola grapes from three wineries (Poli, Pedrotti, and Pisoni in the Italian Alps), indicated that a winery-specific "microbial-terroir" contributed mostly to the wines' microbial community shaping, rather than a regional "terroir" [16]. As a result of the spontaneous fermentation, the complex microbial diversity which composed the grapes' microbiome, including *Aureobasidium pullulans*, *Starmerella meliponinorum* MS 2010, *Penicillium polonicum*, *Pichia membranifaciens*, *Candida zemplinina*, *Penicillium bialowiezense*, and *Candida ethanolic*, was limited to some specific wine yeast species, which existed in limited relative abundance before fermentation, such as *Saccharomyces cerevisiae*, *Pichia membranifaciens*, and *Hanseniaspora osmophila*. Even though the must from the different wineries had significantly different mycobiome, the dominant presence of *Saccharomyces* at the end of fermentation was observed in all must tested, except from the Poli must, in which *Hanseniaspora osmophila* was also dominant.

6. Combination of microbial evolution studies with metabolism analysis could provide indications of the microbial terroir

The different varieties of grapevine (*Vitis vinifera* L.) are differentiated by a unique pool of compounds or chemical precursors that influence the aromatic composition of the produced wines. For instance, linalool is a typical characteristic aroma of Muscat varieties, while methoxypyrazine derivatives characterize the varieties Sauvignon blanc and Cabernet Sauvignon [64]. Apart from the grapevine variety, the degree of ripening, as well as the agronomic and oenological techniques applied, influence also wine's aromatic profile [65–71]. The metabolic reactions performed in wines, due to the specific enzymatic activity of selective wine yeasts that assist to the catabolism of sugar molecules and other ingredients, in order for the aroma compounds to be released have been reviewed extensively [72–74]. Indicatively, the basic yeast enzymes implicated in flavor compounds' secretion from the catabolism of grape components include: (a) glycosidases, such as α -L-arabinofuranosidase, α -L-rhamnosidase or β -D-apiosidase and β -D-glucosidase, which lead to the release of aromatic compounds found in the

bound aroma sections of diglycosides, glucosides and chemical compounds including terpene diols, terpenols, C₁₃-norisoprenoids [72, 75, 76]. These enzymes are produced mainly by the genera *Saccharomyces*, *Debaryomyces*, *Candida*, *Hanseniaspora/Kloeckera*, *Metschnikowia*, *Zygosaccharomyces Kluyveromyces*, *Pichia*, *Schizosaccharomyces* and *Saccharomycodes*, *Brettanomyces*, *Torulaspora* and *Trichosporon* [70, 77–91]. (b) Carbon-sulfur lyases, that catalyze the release of volatile or varietal thiols from glutathionated thiol precursors produced by yeasts, including *S. cerevisiae*, *Pichia kluyveri*, *Candida zemplinina*, *Metschnikowia pulcherrima*, *Hanseniaspora uvarum*, *Kluyveromyces thermotolerans* and *Torulaspora delbrueckii* [92–94].

A great influence on the pool of the VOCs released in wine is due to the metabolic activities performed mostly by predominant yeasts, leading to secondary metabolites' production during fermentation [92]. These secondary aroma compounds include ethanol, CO₂, and glycerol, as well as volatile fatty acids, such as acetic acid and propanoic and butanoic acid esters, higher alcohols and aldehydes, and volatile derivatives of fatty acids and nitrogen- and sulfur-comprising compounds, which have greater contribution to the secondary aroma profile [96–99]. The spontaneous fermentation is conducted by autochthonous yeasts, which exist naturally on the surface of grapes. Increased biodiversity of yeast strains leads to elevated content of VOCs in wine [57]. The majority of the fermentative aroma metabolites are characterized by elevated sensory thresholds [70]. As a result, their combination shapes the characteristic aroma of wines. Importantly, some metabolic reactions performed by must microbiota are considered undesirable, since they spoil the quality of wine, such as by the acetic acid production [95]. Botrytized wine fermentations were found to contain increased abundance of acetic acid bacteria (AAB) in comparison with unaffected wines [36, 64]. Based on that, the selective microbial communities which are related to specific grape varieties, originated from particular locations, may extract distinctive metabolites, the combination of which could provide a characteristic *terroir* to the region [57].

The understanding of the contribution of the microbial communities in the sensorial characteristics of the wine requires the combination of metagenomic studies that will allow the identification of the wine's microbiome, with transcriptomics or metabolomics, which will reveal the volatile profile of the produced metabolites. Bokulich and colleagues [12] proposed that by identifying the microbial pool which composes grapes, and based on the existed knowledge, a great amount of the produced in the wine metabolites could be predicted. Indeed, by applying metabolomics and associating them with microbial communities—metagenomics—they discovered marker metabolites able to differentiate AVAs. Additionally, through a statistical model, they suggested that the grape must microbial conformation is able to predict the metabolites comprising the produced wine, proposing that regional microbial composition patterns may be able to characterize the wine physiognomies. Similarly, Belda and co-workers [96] suggested that the enzymatic activities of the wine-related microbial species population may predict the influence of the produced metabolites on wine aroma and establish region-derived clusters, via combination of metagenomics with information extracted by species-related enzymatic profiles analysis. Through gathering numerous non-*Saccharomyces* yeasts derived from three wine appellations in Spain and relating phylogenetic data with specific wine-associated enzymatic capabilities from glycosidases (β -glucosidase, α -L-arabinofuranosidase and β -D-xylosidase), β -lyases, pectinases, proteases, cellulases and sulfite reductases, indicated distinct origin-associated clusters for species such as *A. pullulans*, *T. delbrueckii*, *W. anomalus*, *H. uvarum* and *L. thermotolerans*.

Importantly, genetic variations among microbial strains may alter the overall profile of the wine's volatiles, proposing the influence of another contributing factor

to regional characteristic terroir. Genetic variances between *S. cerevisiae* strains lead to alterations in the wines' metabolic profile affecting their sensory qualities [100–105]. Fluctuations in the expression levels of key enzymes affecting wine's aroma among different *S. cerevisiae* strains isolated from diverse geographic areas of New Zealand indicated correlations among geographic region and genetic background as well as the phenotypic profile of *S. cerevisiae* [103]. However, the phenotypic plasticity of *S. cerevisiae* to produce altered phenotypes based on the fermentation microenvironment was found to affect the metabolic profile of wines [104].

Moreover, genotypic characterization of different strains of *O. oeni*, isolated from diverse geographic regions during the process of malolactic fermentation, revealed a highly diverse genetic background among the strains derived from different locations, but also strains categorized in the same phylogenetic group were detected in diverse regions, adapted in the same type of wine [105]. Noteworthy, the genomic, transcriptomic, and proteomic profile of various *O. oeni* strains was found to be strongly influenced by microenvironmental conditions during winemaking [106–108].

Brettanomyces bruxellensis (or *Dekkera bruxellensis*), a yeast implicated in wine spoilage producing volatile phenols that create unpleasant flavors, was found to be composed by strains with differences in their genetic background that affected their adaptation in the wine-producing environment [109–112]. Microsatellite analysis of 1488 *B. bruxellensis* strains isolated from diverse geographic locations identified that the *B. bruxellensis* population was differentiated not only based on ploidy level, culture method, and fermentation environment but also on the origin of isolation [112], highlighting again the influence of geographic region in combination with additional influencing factors to microbial terroir formation.

7. Conclusion

Regional characteristics such as climate, agronomic practices, grape variety, and soil chemistry may influence the composition of the local microbial communities creating a characteristic regional microbial profile described with the term “microbial terroir.” The composition of a particular variety grape microbiome, beyond its dynamic fluctuations during fermentation, was found to be able to provide indications regarding the chemical composition and the sensorial characteristics of the produced wines. The existence of specific regional microbial biomarkers, able to predict the metabolic composition of the wine, is a powerful indication of the existence of a clear association between region and local microbiome. Future studies based on the combination of HTS technologies with metabolomic studies may provide more enhanced evidence regarding the contribution of the regional microbial communities to wines' sensorial characteristics.

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