

The concept of terroir: what place for microbiota?

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Abstract

Microbes play key roles on crop nutrient availability and on plant growth and health. Recent advances in technologies, such as High Throughput Sequencing Techniques, allowed to gain deeper insight in the biodiversity of microbial communities associated with soil, rhizosphere and plant phyllosphere. Over the past 10 years, numerous scientific studies have been carried out on the microbial component of the vineyard and agree on the evidence of regional delineations of microbial communities, that may contribute to regional wine characteristics and typicity. Some authors proposed the term “*microbial terroir*” including “*yeast terroir*” for grapes to describe the connection between microbial biogeography and regional wine characteristics. Many factors are involved in terroir including climate, soil, cultivar and human practices as well as their interactions. Studies considering “*microbial terroir*” greatly contributed to improve our knowledge on factors that shape the vineyard microbial structure and diversity. However, the potential impact of “*microbial terroir*” on wine composition has yet not received strong scientific evidence and many questions remain to be addressed, related to the functional characterization of the microbial community and its impact on plant physiology and grape composition, the origins and interannual stability of vineyard microbiota, as well as their impact on wine sensorial attributes.

Introduction

According to the International Organisation of Vine and Wine definition (resolution OIV/VITI 333/2010), vitivinicultural *terroir* is a concept which refers to an area in which collective knowledge of the interactions between the identifiable physical and biological environment and applied vitivinicultural practices develops, providing distinctive characteristics for the products originating from this area. *Terroir* includes specific soil, topography, climate, landscape characteristics and biodiversity features. The idea that microbial activity is part of *terroir* is old, but paradoxically little studied until recently. This idea was relayed by empirical observations of some wine growers believing that inoculation with selected yeast could reduce or even suppress *terroir* expression in wine. The pioneering study of Bokulich et al., (2014) proposed a connection between microbial biogeography, referring to special distribution of biodiversity, and regional wine characteristics. These authors termed this connection as “*microbial terroir*”. Since then, a consistent number of articles has been published each year on “*microbial terroir*”, among which 5 reviews, with a marked increase in 2021 (Figure 1). In parallel, the concept of microbial *terroir* has also been the subject of contradictory results, in particular for *terroir* yeasts and has been questioned. The aim of this presentation is to give a short overview on the role of microbiota as a *terroir* component and to highlight future perspectives and challenges on this key subject for the wine industry.

Microbiota from vineyards to wine

Microbiological community studies have largely benefited from the development of Next-Generation Sequencing technologies (NGS) that enable the detection of microorganisms present in different vineyard compartments (soil, rhizosphere, trunk, leaves, flowers and fruits) with the aim to gain new insight into the biodiversity and structure of microbial communities. Microbial composition is generally addressed by means of 16S rRNA gene and ITS (Internal Transcribed Spacers) NGS for elucidating bacterial and fungal communities, respectively.

Vineyard microbiota, mainly fungal, bacterial, archeal and yeast populations, plays an important role in soil productivity, plant nutrition and health. Microbiota, as part of bulk soil, is integral to biogeochemical cycles and directly impacts soil fertility and chemistry. In the rhizosphere directly surrounding plant roots, soil microbiota acts as a microbial reservoir and interacts with plant health, physiology and phenotype (reviewed by Griggs et al., 2021 and Darriaut et al., 2022). Plant compartments harbour distinct microbiota; leaves, flowers and fruits host fungal and bacterial communities, and the main source of these microorganisms was reported to be vineyard soil (Zarraonaindia et al., 2015; Ramírez et al., 2020). Atmospheric microbiota is another contributor to the fungal communities of the aboveground organs (flowers, fruits, and leaves) and *vice versa* (Abdelfattah et al., 2019). The structure and function of fruit microbiota strongly change over the season with plant phenological development and the availability of substrates in exudates. At maturity stage, grapevine associated microbiota is at the origin of must microorganisms. Due to the strong modifications of the environment during the crushing of the berries, only few bacterial and yeast species are able to adapt to the grape juice composition (low pH, high sugar content, SO₂, lack of oxygen, ...), to initiate a growth and to be actors of the wine fermentation. Hence, a strong decrease in biodiversity occurs from vineyard to wine, which is accompanied by some strong modification of the microbial composition and structure.

Evidence for the existence of microbial biogeography both at the community and population levels

Geography is traditionally considered to have a low impact on microbiota distribution due to large population sizes and limitless dispersal abilities (Knight et al., 2020). However, an increasing number of scientific studies conducted in different vineyard compartments and at different scales (world, countries, regions and vineyards) gave evidence for the existence of biogeographic differentiation of microbial community or population (Table 1). The first evidence of regional distribution of grape berry and must bacterial and fungal communities has been revealed by the pioneering study of Bokulich et al. (2014), relayed by Gilbert et al. (2014). These authors demonstrated that microbial biogeography is nonrandomly associated with regional, varietal, and climatic factors across multiscale viticultural zones. Then, the following studies reported similar results in other wine producing regions: in Chile (Jara et al., 2016 ; Miura et al., 2017), Italy (Vitulo et al., 2019), China (Li et al., 2021) and Greece (Chalvanti et al., 2021). In vineyard soil microbial communities, geography was also showed to determine microbiota in line with results obtained for grape must compartment (Oyuela Aguilar et al., 2020); a link between spatial distance and microbial community on global and regional scale was confirmed (Gobbi et al., 2020) and even proved to be valid at local scale (Knight et al., 2019). Bacterial and fungal communities present in soils were shown to be affected by the soil physical and chemical properties. In a recent investigation using a national soil survey, the bacterial and archaeal phyla present in the soil were assayed by pyrosequencing approach targeting 16S rRNA gene with the aim to identify the main environmental drivers of phyla distribution (Karini et al., 2018). As it was previously reported on world wide, regional and landscape scales, pH and land management were the main recurrent drivers, followed by soil texture, nutrients and climate, but a significant amount of variance remained to be explained.

At population level, the main fermentative yeast species *Saccharomyces cerevisiae* was often considered. There is evidence of population differentiation at distance of over 100 km and even at global scale, with geographically isolated lineages (Gayevskiy et al., 2012 ; Liti et al., 2009) but not at vineyard scale (within 2-10 km radius) (Börlin et al., 2016 ; Knight et al., 2020). At local scale within vineyards, insects like bees, wasps and fruit flies as well as birds and human activities are likely vectors of *Saccharomyces cerevisiae* at the origin of its dissemination in the environment. Consequently, the recurrent presence of native yeast strains specific to a given vineyard, which is present only in this area (“terroir yeast”), has not been demonstrated so far.

From microbial biogeography to microbial terroir: limits, challenges and future perspectives

To evidence a possible link between soil microbiota and terroir expression in wines, studies addressing the *inventory* of the existing microbiota should be completed with *functional* approaches. Major drivers of terroir expression are air temperature, soil temperature, radiation, soil water availability and soil nitrogen availability (van Leeuwen et al., 2018). Among these, vine nitrogen status is highly dependent on soil microbiological activity, involved the mineralisation of soil organic matter (Verdenal et al., 2021). Hence, studies on functional soil microbiology focussing on terroir expression in wine production should primarily focus on microorganisms involved in the nitrogen cycle.

To definitively consider the microbial component as part of the *terroir* concept, a significant link between fermentative microbiota and sensory regional distinctiveness and wine style should also be established. As preliminary results, association between grape must and wine microbial community and/or population and wine metabolic fingerprint were highlighted by Bokulich et al., (2016) and Knight et al. (2015). However, in both studies, no sensory analyses on wines were performed. To assess whether different vineyard microbiota could significantly modulate wine sensorial properties and typicality, controlled experiments combining microbiota analysis, vine nitrogen status, as well as wine chemical and sensory analysis should be implemented.

Until now, vineyard microbial biogeography was established on the basis of High Throughput Sequencing which is relevant to establish an exhaustive overview of the microbial biodiversity. However, different biases have been described associated to metagenomic analysis by High Throughput Sequencing in link with DNA extraction method: presence of interfering agents for molecular analysis, defining the appropriate balance between amplifying all members of every taxon with high coverage and having the highest taxonomic resolution as possible (Belda et al., 2017). In the context of “microbial aspect of the *terroir*”, the major limitation of NGS techniques relies on its inability to address the functional part of the microbial communities. Considering soil, rhizosphere and rootstock compartments, a change in the structure/diversity of the microbiota does not necessarily mean a change in functions (Marasco et al., 2018). In case of wine fermentations, the analysis of gene diversity related to growth kinetics, fermentative metabolisms and volatile compound synthesis would be a necessary complement of investigating taxonomic diversity alone. Many previous studies have provided evidence that strains are as relevant as species to be considered when it comes to study the impact on wine metabolome and sensory properties. NGS methods hardly lead to strain level resolution. The resident microbiota of the winery is an important source of microorganisms driving the uninoculated fermentation process. This microbiota originates from vineyard and after the selection and enrichment process through the grape juice transformations into wines, it could be resident in the cellars (Beltram et al., 2002). Should this resident microbiota be considered as part of the *terroir* composition or could it on the contrary alter the expression of *terroir* microbiota?

Another challenge is to consider whether or not the microbial *terroir* persists with inter-annual stability. Most of the studies are conducted over a short time period, generally 2-3 years, and can hardly provide evidence for the existence of a long-term regional microbial signature. The scale considered as *terroir* is another issue; in some vineyards, plots that are separated by only few meters could be considered as different *terroirs* (Alexandre, 2020). Until now, studies taking into account intra-vineyard differences are rare but should be considered to complete our knowledge on microbial biogeography.

Microbial interactions with grapevines at the root-soil interface have the potential to modulate grape composition and subsequently wine chemical and sensorial characteristics. Further studies, combining taxonomic and functional levels, are needed to assess the indirect effect of microbial *terroir* on wine quality by impacting vine health and physiology. Microbial biogeography could also be explored in light of biotic relationships existing between soil population (bacterial, archaeal and fungal population) through interaction networks and to integrate biotic and abiotic interactions.

The concept of *terroir* includes climate, soil, cultivar, human practices and the interaction between these factors (van Leeuwen and Seguin, 2006). The microbial assembly is a factor that must now be taken into account and it will be important in the future to assess its relative contribution to the sensory regional distinctiveness of the wines in comparison to the other factors. In *terroirs* with high quality performance, bio-indicators related to the biodiversity and function of the microbial community would be relevant to define. In the future, research programs would certainly benefit from integrating interdisciplinary approaches (microbiologists, soil scientists, vine ecophysicists, enologists and *terroir* specialists) to address this complex concept. Then additional work is needed before considering that it may be possible, “by inoculating soil microbes on ripe grapes, to reproduce a wine *terroir* in sites *a priori* not suitable to generate that specific wine”.

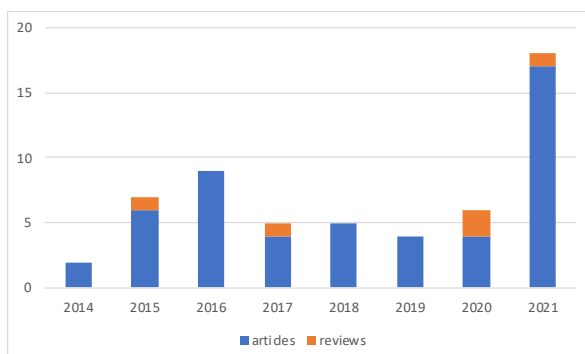


Figure 1. Number of publications (articles and reviews) with “Microbial terroir” related to vineyard and wines (source: Google Scholar and NCBI searching).

Table 1. Examples of scientific studies related to microbial biogeography published since 2014

Scale	Compartment	Microbial community	Main results	Duration of the study	References
Region, California	Grape must	16S rRNA, ITS	Grape-associated microbial biogeography is nonrandomly associated with regional, varietal, and climatic factors across multiscale viticultural zones	2 years	Bokulich et al., 2014
Regions, Chile	Grape berry	Yeast	Microbial terroir might be affected by climatic conditions such as relative humidity and rainfall, especially impacting the load of non-conventional yeast	1 year	Jara et al., 2016
Vineyard, Chile	Leaves, grape berry	16S rRNA, ITS	Leaf and berry fungal community dissimilarities between locations increased with geographic distance, but not for bacterial community	1 year	Miura et al., 2017
Intra-vineyard, New Zealand	Soil, ferments	26S rRNA	Vineyard soil fungal communities were differentiated at local scale but not grape must <i>S. cerevisiae</i> populations	1 year	Knight et al., 2019
Regions, Italy	Bark and Grape berry	16S rRNA	“Microbial terroir” seems to be even more marked in bark than in berries	1 year	Vitulo et al., 2019
Continents Countries region	252 topsoil (0-10 cm), 200 vineyards	16S rRNA, ITS	Geography determines microbiota; can be used to predict the origin of a vineyard’s soil	3 years	Gobbi et al., 2020
Vineyard, Argentina	Soil and root	16S rRNA, ITS	Bacterial and fungal communities present in rhizosphere soils are primarily affected by soil composition	3 years	Oyuela Aguilar et al., 2020
Regions, Greece	Grape juice	Yeast	Yeast communities within an area were temporarily stable, correlated with maximum temperature, elevation, and net precipitation	3 years	Chalvantzi et al., 2021
Regions, China	Grape berry	ITS	A landscape of fungal biogeographical patterns across the main Chinese wine-producing regions was delineated.	1 year	Li et al., 2021

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