BIO Web of Conferences **9**, 01012 (2017) DOI: 10.1051/bioconf/20170901012
40th World Congress of Vine and Wine

WineSeq®: A new tool for the study of the functional biodiversity of soils, and its use as a biomarker and guide for vitiviniculture practices

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Abstract. Apart from their explicit viticulture value, vineyards are natural reservoirs of biological diversity, constituting a complex, but interesting, anthropogenic ecosystem. The functional biodiversity found in vineyard soils is determinant not only for the physical-chemical and nutritional properties of these soils, but also for vine health and grape yield and quality. Diseases affecting vine health or grape quality cause significant economic losses for wineries, specially in the case of old and unique vineyards. In a precision viticulture context, the use of rational phytosanitary treatments and the application of adequate agronomical practices is the only way to maintain the biodiversity required by the fields to keep their stability and resilience. In this context, we have developed WineSeq® (`https://wineseq.com/`), as the first online portal created to support the management of vine health and yield and to prevent or to diagnose vine diseases. The portal (`https://portal.wineseq.com/`) shows filtered and interpreted results of the global microbial population analyzed by metagenomics (DNA Next Generation Sequencing) from soil, wood, grapes or fermentation samples. WineSeq® technology is based on machine learning and cloud computing to integrate microbiome information with climatic, edaphic and agronomical information of interest. Thus, we make easier and understandable the analysis of the microbial variable for vinegrowers and winemakers.

1. Introduction

In 2015, the total world area under vines reached 7.534.000 ha. More than a half are located in the European continent, with Spain topping the list (1.021.000 ha), followed by France (786.000 ha) and Italy (682.000 ha). Outside Europe, China (830.000 ha), Turkey (497.000 ha) and USA (419.000 ha) are the biggest vineyards; the planted surface in Argentina (225.000 ha), Chile (211.000 ha) and Australia (149.000 ha) should also be highlighted. Regarding wine production, it reached 274.400 hl worldwide in 2015, standing out Italy (49.500 hl), France (47.500 hl), Spain (37.200 hl) and US (22.100 hl) [1].

Different countries establish national legislations to guarantee the sustainability of vineyard plantations and replacements, and also, together with regulatory councils, they establish quality requirements in both viticulture and enology practices with the aim of defining quality standards. In this regard, wines with distinctive autochthonous peculiarities have a great acceptance among consumers, causing important economic consequences, and Apellations of Origin are looking for distinctive markers of their terroir. However, the main heritage of wine regions are their old and genuine vineyards, in which a lot of time and money have been spent by vine growers to develop high quality grapes that produce recognizable and high-quality wines [2,3].

Thus, preserving the health of vineyards is the only way to guarantee their future life, and for that purpose it is necessary to understand the basis of vine diseases and to control and prevent their development in the field. Two groups of diseases are in the spotlight due to their economic consequences: vine trunk diseases and grape rots, being the former those that threaten the vineyard heritage and the later the ones that cause short-term losses in wine quality and production.

We can define 33 key vine diseases with microbial origin [4], where those affecting long-time organs (trunk, roots, etc.), like Esca, eutyposis or *Botryosphaeria* infection are the main ones related with trunk and could affect both old and new plantations. Other diseases such as Black foot or Petri diseases are more specific for young vineyards, threatening their longevity and productivity. Altogether, trunk diseases cause economic losses of about 1.500 million dollars per year [5,6].

The substitution of the affected vines with new plantations, together with an adequate disinfection treatment of soils, used to be the most definitive way to stop the spread of an infection; however, it implies substantial investments of money [7]. Table 1 shows that, compared with the cost of keeping rigorous diagnostics and preventive and palliative actions in vineyard management (estimated in 600–1.000€ per Ha and year), the economic consequences of a diseased vineyard could overcome 300.000€ considering the cost

Table 1. Mean calculation of the economic losses associated with a vineyard death and replantation.

Cost of replacement of diseased vines		
Vine cost (original and replacement)	1,20€/vine	2,40 €
Planting and maintenance costs (first 5 years)	4€/year	20 €
Total cost per vine		**22,40 €**
Vines/Ha	1.500	
Total cost per Ha		**33.600,00 €**
Economic losses in wine production		
Kg of grapes per vine	4	
Kg of grapes per Ha	6.000	
Bottles per Ha	6.000	
Cost per bottle	9,00 €	
Indirect losses per year		**54.000,00 €**
Years until ideal productivity	5	
Total losses		**270.000,00 €**

of vines replacement and the losses from wine miss-production.

Due to the evident economic consequences of vine diseases, different solutions have been proposed to monitor and diagnose the phytosanitary conditions of vineyards. As an example, the use of multispectral aerial images, taken by satellite or by drones, are in the spotlight of these approaches. However, the difficulty of the treatment of vine trunk diseases when external symptoms appear, do not allow the use of these image-based technologies with early diagnosis objectives.

WineSeq® is the first online platform designed to prevent and diagnose vine diseases, based on the study of the complete vineyard microbiology. It reports the phytosanitary state of the sampled vineyard, with the precise detection of any microbial pathogen of vine or grape, estimating its severity or risk level. WineSeq® technology uses Next Generation DNA Sequencing to identify the entire microbial population (bacteria, filamentous fungi and yeasts) of different samples (soils, wood, grapes, musts, wine, etc.). The vast amount of microbial information is processed by an informatics algorithm based on machine learning to integrate it with climatic and Geografical Information to create a robust model that makes it possible to define the epidemiology of diseases, the effectivity of agronomical practices, the existence of cross-contaminations (fecal, landfill, etc.), and to assist in zonification works.

Based on this technology, we developed WineSeq® Portal (`https://portal.wineseq.com`) as the online platform where winegrowers and winemakers can explore the microbiome of their vineyards. This Portal has been created to easily understand the origin of vine diseases or potential wine inocula.

2. Material and methods

2.1. Sample collection

2.500 soils and 635 grapes were sampled from wineries of 18 countries during 2015 and 2016 (Fig. 1).

All the samples were collected according to a methodology standardized with bibliography and our own

Figure 1. Worldwide distribution of soil samples contained in WineSeq database.

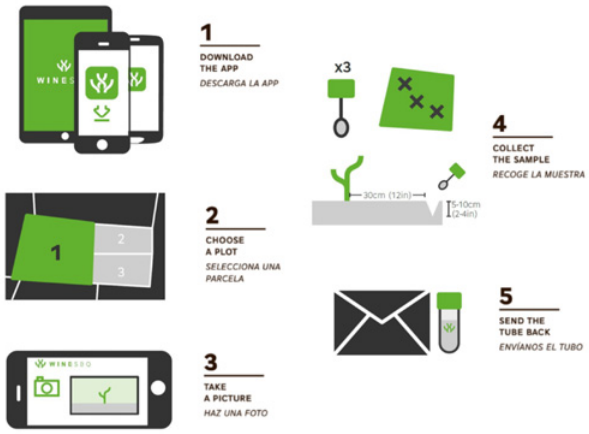

Figure 2. Sample collection instructions for WineSeq analysis.

experience [8], and with the aim of making the work for the winegrower simple (Fig. 2):

- Plot selection: a plot is defined as a vineyard surface with homogeneous soil properties, the same grape variety and managed with the same agronomical practices. If a plot is located in a slope higher than 7%, it should be divided in 3 zones: upper, middle and lower.
- Sample collection: winegrowers received a labeled sterile tube that is scanned with WineSeq App® available for Android and iOS mobile devices. Three spoons of soil should be collected from 3 points randomly selected in the plot. The exact point for soil collection should be at 30 cm from the vine (onto the lane) and at 5–10 cm deep from the first untilled fraction of soil.
- Metadata: information of interest, such as the location of the vineyard, is collected by taking a picture of the sampling point, using WineSeq App®. Additional data (grape variety, agronomical practices, etc) are collected by a digital form completed by WineSeq App® or online Portal.
- Samples are sent by regular courier service and received at WineSeq laboratories within 24 h.

2.2. Microbial population analysis

Nucleic acids are extracted directly from samples, using our own extraction methodology that avoid biases,

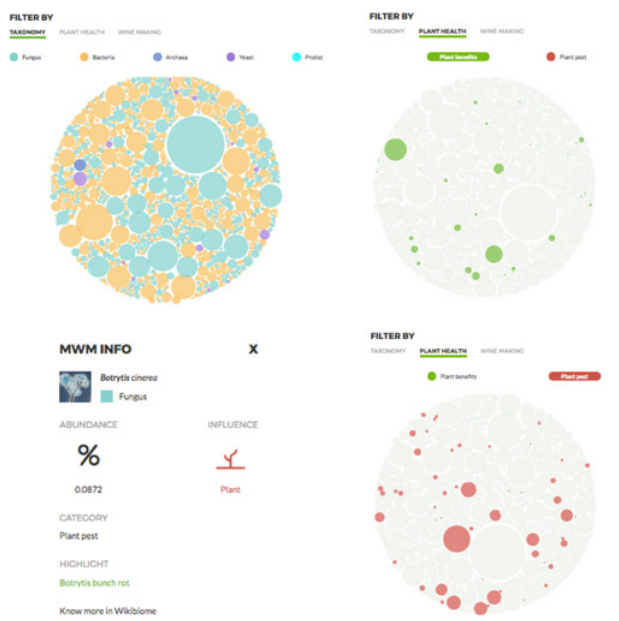

Figure 3. Microbiome exploration tool available at WineSeq portal. Different images are included: complete taxonomical composition of microbiome (upper left); information of beneficial microorganisms for vine health (upper right); summary information of a specific pathogen detected (*Botrytis cinerea*) (bottom left); information about detrimental microorganisms for vine health (plant pests) (bottom right).

allowing us to detect the maximum possible diversity values. Next, taxonomic-marker genes for both prokaryote and eukaryote microorganisms are amplified using oligonucleotides designed and patented by Biome Makers Inc. Each amplicon sequence obtained from the PCR is sequenced using Next Generation Sequencing (Illumina technology).

As a mean, 300.000 reads of DNA are obtained per sample that are then analyzed using a meticulous bioinformatics pipeline, also protected under patent. In brief, DNA reads are clustered by their sequence similitude in Operational Taxonomic Units (OTUs). Each OTU is compared with a taxonomic database, developed and curated from international databases (such as SILVA, GreenGenes, etc.), making possible the later taxonomic assignment to each sequence cluster. The abundance of each taxonomic group is calculated as a percentage of the total amount of OTUs detected, and the distribution and functional diversity of the population is represented in an intuitive tool, available at Wineseq® Portal (Fig. 3).

2.3. VERITAS

Genomic data and the metadata associated to each sample are collected in an intelligent database composed by 119 different information boxes. For example, the 'climatic box' contains several parameters collected by connecting to the climatic station closest to the sample point. Every data, both genomic and metadata, are integrated and interconnected with machine learning algorithms, generating significant correlations and risk estimations within the disease prevention model. As an example of the connection of algorithms, the presence of powdery mildew, downy mildew and botrytis pathogens are crossed with the previously known algorithms of climatic risk for these diseases.

2.4. WineSeq® portal

WineSeq® portal is the online platform that translates the information generated by VERITAS (coming from the previous microbiome analysis) in simple, understandable and intuitive results and conclusions of vitiviniculture relevance. This portal is divided into a private zone, composed by the reports of the samples analyzed (regarding vine health, fermentative microbiota, and microbiome exploration tools) and a public community zone, with open access to the registered users of the 'WineSeq® community' (`https://community.wineseq.com`). This section contains a WikiBiome, as a repository of biological and viticulture/enological information of relevant species for vine health and nutrition and for the winemaking process. WineSeq® community, is supported by a group of international experts (from industry and academia) and it is conceived as a dynamic source of information coming from the questions and answers shared in the open forum by the users of the portal (always moderated by experts).

3. Results

The study of the microbiome of more than 2.500 vineyards, allowed us to extract some relevant conclusions about the frequency and virulence (that is, about the epidemiology) of diseases affecting vine health and grape quality. Soils are the main reservoir of vineyards' microbiome [8] and their biodiversity interacts positively and negatively with the plant and the later fermentative processes [9]. Thus, analyzing the entire microbiome of the soils makes it possible to detect the presence of pathogenic bacteria and fungi (as the basis of the precise application of phytosanitaries), but also to unveil their nutritional potential by identifying microbes related with nitrogen or phosphorous bioavailability and their future stability when detecting biocontrol agents or elicitor-producing organisms.

Figure 4 shows a comparison of the relative abundance of some vines and grapes diseases detected in soil and grape samples. In the case of trunk diseases, all of them are detectable in soil samples, but in certain cases (such as crown galls) they are not found in any grape samples. This fact, together with a precise detection of grape diseases/rots, males soil samples the most adequate matrix to develop general studies of the vineyard microbiome. In addition, and being aware that soil is a dynamic ecosystem, it could be considered as the most stable part of the vineyard along the year, since the development of periodical analyses confirms the consistence of the study of microbiome in soils.

Thus, the analysis of soil microbiomes, has emerged as a useful tool for the monitoring of the health status of vineyards, making possible the implementation of precision viticultural practices focused on the functional biodiversity of soils.

An early detection of microbial pathogens allows for an efficient and targeted intervention to detain the development of the specific infectious agents risking the health of the vineyard. Figure 5 shows a comparison of

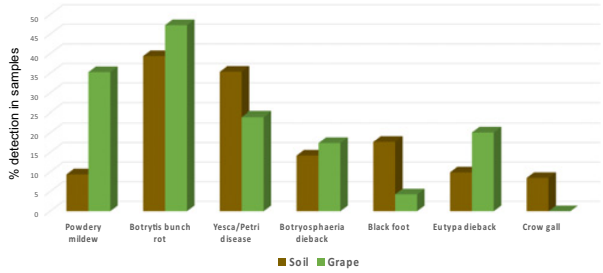

Figure 4. Frequency of detection of vine and grape diseases in soil vs. grape samples.

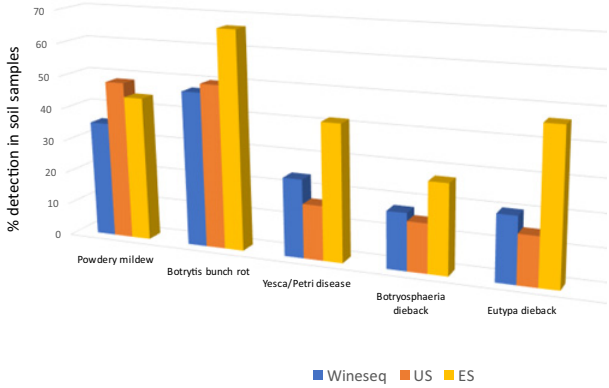

Figure 5. Frequency of detection of vine and grape diseases in soil samples from US, Spain (ES) and from the worldwide WineSeq database.

the frequency of diseases in vineyard soils from Spain and USA (Napa Valley). The main conclusion of this analysis is that, regarding trunk diseases, Spanish vineyards appear to be more susceptible. This fact could be determined by the different viticulture strategies that are commonly applied in new-world *vs* old-world vineyards, and also by the differential susceptibility of the grape varieties and clones used in both continents. These conclusions are enough evidence to start the establishment of a global strategy against trunk disease, based in the knowledge of the epidemiology trends, the edapho-climatic determinants and the biodiversity context of these diseases, with special focus on saving the future of genuine *terroirs* and old vineyards.

Figure 6 shows a partial visualization of the summary report from a soil microbiome analysis in WineSeq® Portal. Within the vine health report, there is simple information about the detection or not of the main 33 vine/grape diseases (Table 2) and, in the case of a positive detection, three additional pieces of information are reported: severity (estimated by pondering the abundance of the pathogenic microorganism with the mean abundance level of this pathogen in healthy samples and with the virulence of the specific microorganism detected causing this disease), frequency (as the global frequency of detection of this disease in our database) and the responsible microorganism (as specific information on the pathogenic agent detected in a sample among the diversity of microorganisms involved in a single disease).

The severity level of a disease detected ranges from Mild to Aggressive, being the complete absence of the pathogens causing a disease informed as "not detected". Furthermore, for every reported disease, there are available

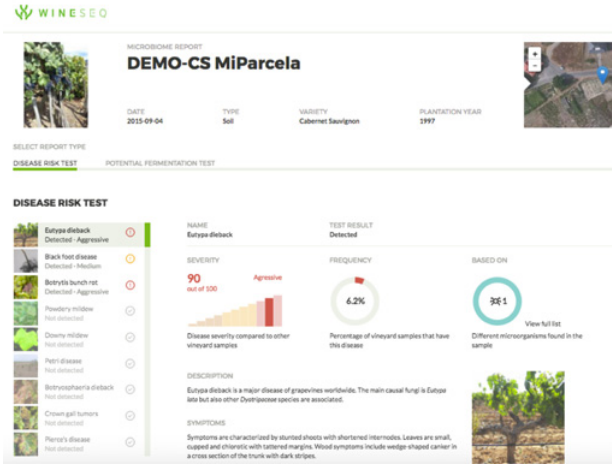

Figure 6. Disease risk test report (WineSeq Portal) from a model sample containing three diseases: Eutypa dieback (with aggressive presence); Black foot disease (with Medium risk); and *Botrytis* brunch rot (with a high risk). It is also shown the specific brief report for the Eutypa dieback detected, including the severity grade, the worldwide frequency of this disease and the number of microorganisms causing this disease in the sample. In addition, a brief description of the disease and its symptoms is included in the bottom part of the image.

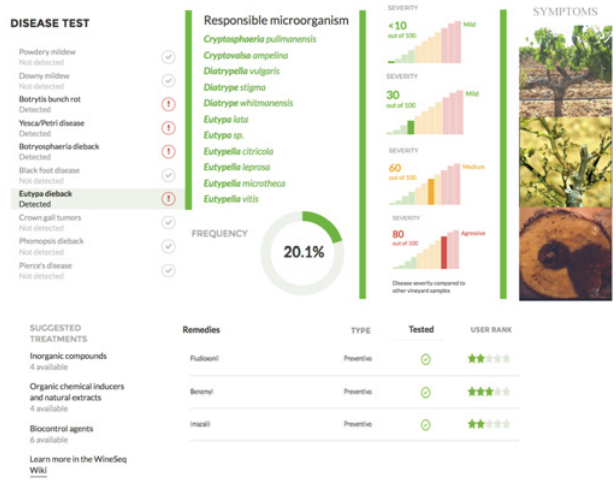

Figure 7. Information available at WineSeq Portal for a disease detected in a sample analyzed by WineSeq.

images of the symptoms, making easier their identification in plants or fruits. For each disease present in a sample, WineSeq® Portal contains information and recommendations about treatments organized in sections depending on their origin: 'inorganic compounds', 'organic chemical inducers/natural extracts' and 'biocontrol agents' (Fig. 7).

As a mean, each vine disease can be caused by 5 different microbial species. Some important diseases with a single origin are downy mildew *(Plasmopara viticola),* botritis infection *(Botrytis cinerea)* or Pierce disease *(Xylella fastidiosa)*. On the other hand, there are diseases with a complex microbial origin, such as esca (up to 40 related species) or the black foot disease (up to 13 related species). In these cases, the specific species involved in an infective process can cause different symptomatology, infection virulence and spreading, and thus, the treatment should be selected and applied with precision.

Table 2. List of important vine/grape diseases with microbial origin, and summary of the microbial groups with enological relevance.

Main vine/grape diseases	**Microbial groups with enological relevance**
- Alternaria rot	- Lactic acid bacteria
- Brenner Rot	- Acetic acid bacteria
- Angular leaf spot	- Conventional starters (*Saccharomyces* and *Oenococcus*)
- Anthracnose	- non-*Saccharomyces* yeasts
- Armillaria root rot	- *Brettanomyces*
- Grape rots (*Aspergillus, Botrytis*)	- Fermentation problems •Refermentations (*Zygosaccharomyces)* •Fermentation stucks (*Lactobacillus kunkeei)*
- Bacterial blight	
- Bitter rot	
- Black foot disease	
- Black rot	
- *Botryosphaeria* dieback	
- Crown gall tumors	
- *Dematophora* root rot)	
- Downy/powdery mildew	
- *Cladosporium* leaf spot	
- Esca / Petri disease	

Apart from the vine health report, Wineseq® provides an additional report with information about 'potential fermentative microorganisms' found in the vineyard. This report contains information about relevant bacteria and yeast species for the winemaking process (Table 2). Since the correlation between soil, grape and must microbiomes have been demonstrated by robust scientific works [8], with the aim to foresee the wine microbiome from vineyard samples we have developed an algorithm based in the survival rate of microbial species detected along entire the production line (soil-grape-must-wine) in both organic and conventional vineyards. The information contained in this report could be used to guide the individual management of grapes from each vineyard in cellar, developing high-risk (but appreciated by consumers) enological practices such as spontaneous fermentations or low sulphite wines with a previous knowledge about fermentative risks and enhancers found in different vineyards.

4. Conclusions

The health status of vineyards should be controlled to guarantee the quality of the later winemaking process. Precision viticulture pursues the control of all the variables determining the quality of grapes, and thus the microbial determinants of vine health and wine quality should be understood. This knowledge will make it possible to develop precise and eco-logical viticulture and agronomical practices (nutrition, tillage, phytosanitary addition…) depending on the microbial potential and risks of vineyard soils. In addition, developing routine microbiome analyses such as WineSeq® that allows for the detection of vine diseases (before symptoms appearance and pathogen spreading) can save high amounts of money derived from the uncontrolled use of phytosanitary products, and even from huge losses caused by vines death and vineyard replacement (Table 1). In this sense, WineSeq® can also be useful to evaluate the quality of vineyards in purchasing processes, accrediting their sanitary conditions or their functional biodiversity.

Furthermore, the vineyard microbiome, as a reflect and biomarker of terroir, can be used as a tool for plots zonification and for the definition of differential management zones or vineyards of special consideration (such as the Spanish "Vinos de Pago").

Massive DNA sequencing technology for soil samples, such as WineSeq®, is the only way to explore the entire microbial community of vineyards. The complexity of the raw results obtained with this avant-garde technology requires a screening and interpretation to extract relevant and understandable information for winegrowers and winemakers. In the case of WineSeq® Portal, it shows a complete but intuitive report with information about diseases affecting vines and grapes and with an estimation of the fermentative potential of the grapes analyzed from different vineyards. Finally, WineSeq® Community has been created as the first worldwide online community to share information, questions and advice about vines health, vineyard management and winemaking trends.

References

[1] OIV, *State of the Vitiviniculture World Market* (OIV, Paris, 2016)
[2] J. Moulard, B.J. Babin, M. Griffin, IJWBR **27** (1), 61–78 (2015)
[3] S. Caple, M. Thyne, (2016) `http://academyofwinebusiness.com/wp-content/uploads/2014/07/Co04_Caple_Sue.pdf`
[4] WineSeq, (2017) `https://portal.wineseq.com/wikibiome`
[5] V. Hofstetter, B. Buyck, D. Croll, O. Viret, A. Couloux, K. Gindro, Fungal Divers. **4**, 51-67 (2012)
[6] D. Gramaje, J. Armengol, Plant Dis. **95** (9), 1040-1055 (2011)
[7] G. Bruno, L. Sparapano, Physiol. Mol. Plant P. **71**, 210–229 (2007)
[8] I. Zarraonaindia, S.M. Owens, P. Weisenhorn, K. West, J. Hampton-Marcell, S. Lax, et al. MBio **6**(2), e02527-02514 (2015)
[9] I. Belda, I. Zarraonaindia, M. Persisin, A. Palacios, A. Acedo, Front. Microbiol. **8**, 821 (2017)