

# Txakoli vineyard's bacterial diversity and composition

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## INTRODUCTION

- Microorganisms are critical for vineyard maintenance and wine production, participating in soil functions and nutrients availability, promoting plant pathogen suppression, and involved in the fermentation process, essential in winemaking.
- Previous research demonstrates that soil bacterial communities are mainly determined by soil pH while grape-associated communities vary with the region, variety and climate. Interestingly it has been demonstrated that vineyards soil and grapes microbiota end up in the winery determining the final product's organoleptic specific characteristics and the "terroir".
- Hondarrabi zuri* variety producing Txakoli, a white wine from the Basque Country (North of Spain), has recently gained popularity due to the wine quality improvements, increasing in both acreages of production and wine consumption, however the microbial diversity and composition associated to this variety is still unknown.

## OBJECTIVES

- To acquire an in-depth understanding of vineyard-associated microbial communities to improve Txakoli viticulture and winemaking.
- To create a comprehensive inventory of the bacteria associated with *H. zuri* vineyards covering the three main Designation of Origin (DO) of Txakoli: Bizkaiko Txakolina DO, Arabako Txakolina DO and Getariako Txakolina DO.
- To determine the main factors behind the shifts on bacterial distribution and abundances in soil and grape communities.

## MATERIAL AND METHODS

### 1. SAMPLE COLLECTION

- N = 41 vineyard plots (22 Wineries)
- Covering three Designation of Origin
- Two years: 2016 and 2017 at harvest
- Soil = Two replicates per plot
- Grape = Three replicates per plot

### RECOVERED METADATA

- Vineyard factors: slope, orientation, altitude, geographical coordinates, conduction system, rootstock
- Climatic factors: total temperature (Ttemp), total precipitation (Tprec)
- Experimental factors: DO, winery, vineyard and year
- Soil edaphic factors: pH, conductivity and moisture
- Must chemistry: pH, Total Acidity (TA), potential alcohol (PA), Yeast-assimilable nitrogen (YAN)

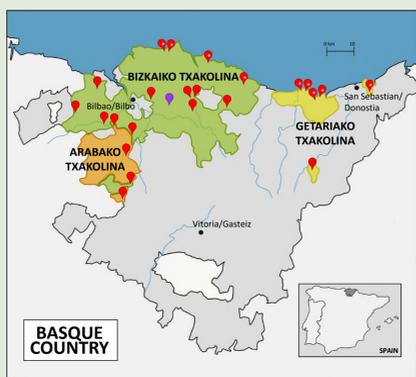


Fig. 1. Txakoli wineries' location. The 22 wineries are represented with red markers. Near-shore vineyards are identified with a white (\*). Unestablished vineyard plot (used as outgroup) is in purple.

### 2. DNA EXTRACTION AND SEQUENCING

#### SOIL

- Power Lyzer Power Soil extraction kit
- Primers 515F and 926R (V4-V5 domain)
- Sequencing on the Illumina MiSeq platform (2x300 bp)

#### GRAPE

- QIAamp DNA Mini Kit (Qiagen, Inc.) extraction kit
- Primers 515F and 806R (V4 domain) + PNA clamps
- Sequencing on the Illumina MiSeq platform (2x150 bp)

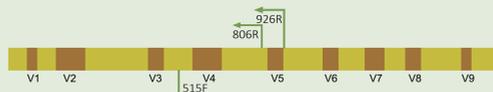


Fig. 2. Graphical representation of sequencing amplification.

### 3. DATA ANALYSIS

- FASTQ raw data were filtered and trimmed (Trimomatic v0.38). Demultiplexing and taxonomical identification were carried out using QIIME 1.9 against SILVA 132 Database.
- Alpha diversity: richness (observed OTUs) and evenness (Shannon index) were calculated in Vegan R Package
- Beta diversity: Bray Curtis index in QIIME 1.9 & Vegan R Package:
  - PCoA (Principal Coordinate Analysis)
  - NMDS (Non-metric Multidimensional Scaling)
  - UPGMA (Unweighted Pair Group Method with Arithmetic Mean)
  - Envfit (Vegan R Package function)
  - CCA (Constrained Correspondence Analysis)
- Compositional differences between groups were identified by Kruskal-Wallis and Lefse (Linear discriminant analysis effect size).

## SOIL RESULTS AND DISCUSSION

- Soil pH significantly correlated with differences in bacterial diversity and composition (Fig. 3 and Fig. 4)

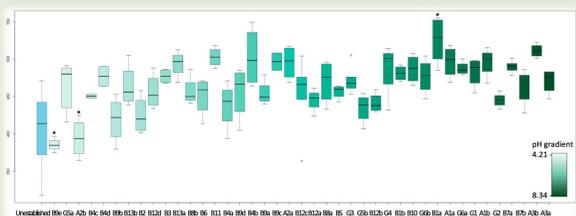


Fig. 3. Vineyards richness boxplot sorted by pH gradient. The vineyards having significantly lower/higher richness (according to Pairwise T-test < 0.05) are marked with \*. The unestablished vineyard (in blue) and the 41 vineyard plots of the study (in green).

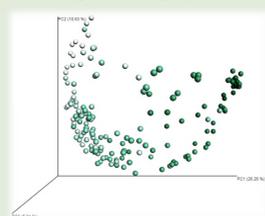


Fig. 4. PCoA plot colored by pH gradient.

- The 41 vineyards clustered into two main groups (Fig. 5, UPGMA Tree) when constrained by pH, year of collection and vineyard. The groups significantly differed in the relative abundances of bacteria associated with:

#### Nitrogen and carbon cycling:

- Branch 1:** *Bradyrhizobium*, *Rhizobium*, *Candidatus Koribacter*, *Candidatus Solibacter*... were augmented
- Branch 2:** Nitrospira, Steroidobacter, Sphingomonas, Chloracidobacteria... were enriched

#### Antimicrobial or pathogen inhibition capacity:

- Myxococcaceae*, *Bacillus*, *Paenibacillus*... preferentially accumulated in **Branch 1**

- Particular trends were observed for vineyards located in near-shore regions (Fig. 5), likely associated to a different microclimate and distinct fertilization regimens compared to inland locations. Historical anthropogenic modifications in land use were found to be relevant in some plots to understand bacterial variation at an intra-vineyard level.

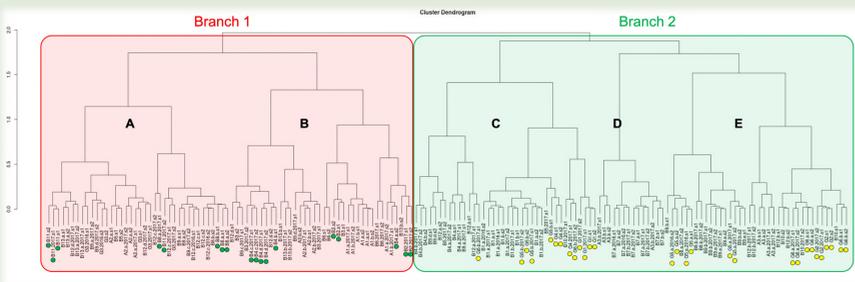


Fig. 5. UPGMA tree clustered on CCA distances constrained by the significant factors resultant from a forward selection analysis (vineyard, year and pH). Samples clustered into two main branches. Near-shore vineyards belonging to Bizkaiko Txakolina DO are labeled with a green dot, while those belonging to Getariako Txakolina are in yellow.

## GRAPE RESULTS AND DISCUSSION

- Differences in grape bacterial richness were found between rootstocks, being the grapes grafted in 41B more diverse and heterogeneous (Fig. 6).

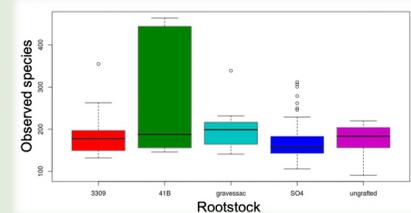


Fig. 6. Richness boxplot by rootstock.

- Samples richness vary by vintage likely due to different climatic and environmental condition governing in each year.

- Bacterial composition differences were also determined by the rootstock *H. zuri* are grafted on, supporting the idea that roots genotype is key for the selective recruitment of microorganisms that afterwards colonize aboveground environments. The abundances of epiphytes with a key role in wine spoilage or fermentation process also differed (Fig. 7):

- Ungrafted** *H. zuri* were characterized by the enrichment of several Alphaproteobacteria (Sphingomonadales, Rhodospirales). Disease causing *Agrobacterium* were found to be augmented, suggesting that this grapes are more vulnerable to tumors.

- 3309** rootstock showed higher abundances of the fungal disease antagonists *Curtobacterium* and *Pseudomonadales* order.

- SO4** rootstock grapes showed an increase in the plant-growth promoting rhizobacteria *Acinetobacter rhizosphaerae*. *Lactobacillus* genera, main malolactic fermentation agents but also wine spoilers at particular conditions, were also augmented.

- 41B** grafted *H. zuri* was augmented in Chloroflexi, likely involved in wine floc formation; Planctomycetes, Nitrospira or Deltraproteobacteria involved in nitrification and nitrogen fixation. Importantly, *Glucanobacter*, key member of must and wine, were also augmented, and positively correlated with grape pH values.

- Gravessac** rootstock grapes showed the indigenous epiphytic *Methylobacterium* and *Phyllobacterium* enriched, contributors of wine final characteristics

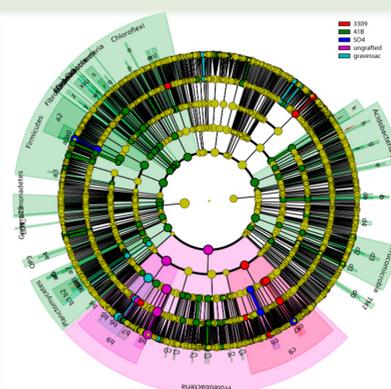


Fig. 7. Lefse cladogram of between rootstock bacterial differences. Kruskal-Wallis  $p < 0.05$ , and LDA scores  $\text{Log}_{10} > 2$ .

41B	3309	gravessac	SO4	ungrafted
o: o_NRP_J	t: o_D5_100	v: c_4_29	c6: o_Enterobacteriales	b8: o_Sphingomonadales
l: c_MBG4	l: c_Differrobacteres	a7: o_Rhodospirales	b1: c_S20	b9: o_Alphaproteobacteria
c: c_MBG8	w: o_Elusimicrobiales	c0: o_S_44	b3: c_Pla4	
d: o_B10	x: c_Elusimicrobia	c1: o_Nitrosomonadales	b6: c_Rizobiales	
e: c_HDRW_WB69	y: o_Ugp450	c2: o_Desulfarcales		
f: o_MV5_40	r: c_Fibrilbacteria	c3: o_Desulfurimonadales		
g: c_BPC102	a0: o_Lactobacillales	c4: o_NK815		
h: c_TMI	a1: o_Turriclacterales	c5: o_Enthomiales		
l: c_Acidimicrobiales	a2: c_Bacilli	c7: o_Pasteurellales		
k: c_Acidimicrobia	a3: c_DPS4	c8: o_Pseudomonadales		
l: o_SBR1031	a4: c_P85_25	c9: o_Gammaproteobacteria		
m: o_SJA_15	a5: c_Q28H05_P_BN_P5	d0: c_S3		
n: o_EnvOP512	a6: c_S07_11	d1: o_Punicococcales		
o: c_Ellin6529	a7: o_MV5_107	d2: o_S_BQ2_57		
p: o_Thermogemmatiporales	a8: o_Cp1a_3	d3: c_Methilacidiphilae		
q: o_S52	b0: o_SH4_13	d4: o_Sediment_1		
r: o_Chroococcales	b2: c_Phycosphaerae			
s: c_Oscillatoriothrixidae	b4: o_p04_C01			

## CONCLUSIONS

- No microbial differentiation between Designations of Origin were found, neither in soil nor in grape consortia, revealing that the Txakoli from the Basque Country viticultural region represents a particular/unique "terroir".
- Soil bacterial distribution is significantly altered by soil edaphic factors (pH), historical land use or distance to the shore, suggesting that past activities and vine location and management practices have a great influence.
- Grape microbial diversity and composition is rootstock specific, consistent with genotype-dependent host colonization success. Interestingly, ungrafted *H. zuri* vines showed higher susceptibility to tumors while grafted vines (such as those grafted on SO4 rootstock) exhibited an increase of particular plant-growth promoting rhizobacteria. The abundances of several genera related with processes in carbon a nitrogen cycles, or antagonist to fungal pathogen colonization also differed suggesting that the rootstock of choice has important implications for *H. zuri* productivity and Txakoli vineyard health
- Overall, this study provides a fundamental background for the yet unexplored diversity and microbial fingerprint associated to *Hondarrabi zuri* vines in the Basque Country viticultural region, unravelling the main factors contributing to microbial shifts.