**PhD DISSERTATION PROJECTS**

**Grape related yeasts as source for designing specific "synthetics microbiota" to be used for wine fermentations.**

Ernesto Petruzziello (ernesto.petruzziello@unina.it)

Dept. of Agricultural Sciences, University of Naples “Federico II”, Italy

Tutor: Prof. Giuseppe Blaiotta

This PhD project aims to highlight the importance of grape microflora in increasing the diversity and improving quality of wines from different *terroirs*. The use of commercial starter cultures (mainly *S. cerevisiae*) reduces the potential of the native microbiota to contribute to the terroir effect. The project involves the development of starter culture design protocol (synthetic microbiota) made up from different selected species from specific *terroirs*. The synthetic microbiota could bring improvements from a sustainability point of view by exploiting the individual characteristics of each selected strains to achieve greater complexity, stability and natural protection of wine.

**I lieviti dell'uva come fonte per la progettazione di specifici "microbiota sintetici" da utilizzare per le fermentazioni vinarie.**

Questo progetto di dottorato mira a evidenziare l'importanza della microflora dell'uva nell'aumentare la diversità e migliorare la qualità dei vini provenienti da diversi *terroirs*. L'uso di colture starter commerciali (principalmente *S. cerevisiae*) riduce il potenziale del microbiota nativo di contribuire all'effetto terroir. Il progetto prevede lo sviluppo di un protocollo di progettazione di colture starter (microbiota sintetico) costituito da diverse specie selezionate provenienti da specifici *terroirs*. I microbiota sintetici potrebbero portare miglioramenti dal punto di vista della sostenibilità sfruttando le caratteristiche individuali di ciascun ceppo selezionato al fine di ottenere una maggiore complessità, stabilità e protezione naturale del vino.

# 1. State-of-the-Art

In recent decades, the biodiversity of the microflora present on the grape surface has been extensively studied, and the main genera of yeasts and fungi present are well known (Castrillo *et al*., 2019). The three most frequently isolated species on the berries surface are *Hanseniaspora uvarum*, *Metschnikowia pulcherrima* and *Starmerella bacillaris*, which also appear to be the dominant species in the must. However, also considering the application of culture-independent (meta-taxonomic) approaches, the real diversity is much higher: 50 species of yeast, belonging to 22 different genera, including *Auerobasidium, Auriculibuller, Brettanomyces, Bulleromyces, Candida, Cryptococcus , Debaryomyces, Hanseniaspora, Issatchenka, Kluyveromyces, Lipomyces, Metschnikowia, Pichia, Rhodosporidium, Rhodotorula, Saccharomyces, Sporidiobolus, Sporobolomyces, Torulaspora, Yarrowia, Zygoascus, and Zygosaccharomyces were identified* (Bokulich and Mills, 2013; De Filippis *et al*., 2017). The diversity and abundance of yeast populations is linked to various factors, in addition to the degree of ripeness of the grapes and the relative variety, the pedoclimate of a specific area, the phytosanitary state of the grapes, agronomic practices and human activities (Wei *et al*., 2022). Currently, there is a greater demand, especially for organic wine producers, to make the best use of the microbiological diversity present in their vineyards. By contrast, the improper and uncontrolled use of non-*Saccharomyces* species could lead to problems both during the fermentation phases and for the final wine quality (spoilage). However, a careful selection of these yeasts could lead to greater complexity, variability and uniqueness of the final product. Although some strains of these species do not have excellent fermentative performances, they can help to improve the sensory attributes of wine thanks to the production of extracellular enzymes and secondary metabolites such as esters, higher alcohols, acids and glycerol. The challenge nowadays is to identify oenologically interesting non-*Saccharomyces* yeasts from different *terroirs*, carrying out an analysis of the metabolic activities of single strains and subsequently evaluating the interactions with other species/strains, then proceeding towards the creation of a synthetic microbiota that simulates spontaneous fermentation. The central theme that climate change has highlighted in enology is certainly the increase in sugars in grapes and therefore a greater quantity of potential alcohol in the final wine, therefore also a lower presence of fixed acids, in fact the use of yeasts non-*Saccharomyces* in co-inoculation, could be an alternative to have a lower ethanol yield, in relation to the quantity of sugars present in the starting must (Castrillo *et al*., 2022).

**2. PhD Thesis Objectives and Milestones**

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **Evaluation of the microbial diversity** of grapes, musts and wines from different *terroirs* (meta-taxonomic analysis).

A2) **Isolation** (culturomic analysis) molecular (identification, biotyping and genomics) and technological characterization of yeasts strains. Genomics analyses will be performed during a laboratory experience abroad.

A3**) Bottom-up,** metabolic activities of individual strains and their interactions.

A4) **Top-down,** design of the synthetic microbiota by lab-scale experiments and use by cellar-scale trials.

A5) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

***Table 1***Gantt diagram for this PhD thesis project.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| *Activity Months* | | ***1*** | ***2*** | ***3*** | ***4*** | ***5*** | ***6*** | ***7*** | ***8*** | ***9*** | ***10*** | ***11*** | ***12*** | ***13*** | ***14*** | ***15*** | ***16*** | ***17*** | ***18*** | ***19*** | ***20*** | ***21*** | ***22*** | ***23*** | ***24*** |
| *A1)* | ***Evaluation of the microbial diversity*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | *Sampling and DNA isolation* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | *Meta-taxonomic analysis* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *A2)* | ***Culturomic analysis*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | *Yeast isolation* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | *Identification, biotyping and genomics* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | *Technological characterization* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *A3)* | ***Bottom-up*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | *Metabolic activities of individual strains* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | *Interactions between strains* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *A4)* | ***Top-down*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | *Lab-scale trials* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | *Cellar-scale trials* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *A5)* | ***Thesis and Paper Preparation*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

**3. Selected References**

Castrillo D, Blanco P (2022) Influence of vintage, geographical location and agricultural management on yeast populations in Galician grape musts (NW Spain). *OENO One* **56**: 65-79.

Bokulich NA, Mills DA (2013) Improved selection of internal transcribed spacer-specific primers enables quantitative, ultra-high-throughput profiling of fungal communities. *Appl Environ Microbiol* **79**:2519-2526.

De Filippis F, La Storia A, Blaiotta G (2017). Monitoring the mycobiota during Greco di Tufo and Aglianico wine fermentation by 18S rRNA gene sequencing. *Food Microbiol* **63**: 117-122.

Rué O, Coton M, Dugat-Bony E, Howell K., Irlinger F, Legras J-L, Loux V, Michel E, Mounier J, Neuvéglise C, Sicard D (2023) Comparison of metabarcoding taxonomic markers to describe fungal communities in fermented foods. *bioRxiv* https://doi.org/10.1101/2023.01.13.523754.

Wei R, Wang L, Ding Y, Zhang L, Gao F, Chen N, Song Y, Li H, Wang H (2022) Natural and sustainable wine: a review. *Crit Rev Food Sci Nutr* **25**:1-12.

Castrillo D, Rabunal E, Neira N, Blanco P (2019) Oenological potential of non-*Saccharomyces* yeasts to mitigate effects of climate change in winemaking: impact on aroma and sensory profiles of Treixadura wines*. FEMS Yeast Res* **19**: foz065.