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**A SELECTION OF YEASTS FOR ENOLOGICAL USE  
IN THE TERRITORY OF DOC ETNA, SICILY**

by Benanti Viticoltori

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## **A Selection of Yeasts For Enological Use in The Territory of DOC Etna**

### ***Introduction***

In the modern winemaking industry the use of microbial starter cultures—selected yeasts cultivated in an active-dry form—is considered an established practice for fermentation. On one hand, the practice guarantees safe and reliable practices that respect a winery's production programs. On the other hand, systemically using imported yeast strains can limit the potential of excellent raw materials and the territorial expression of a wine, contributing to a particular "flattening" of the final product.

In fact, wine expresses the territory in which it is produced. Its characteristics are always the result of a synergy between the influences of Nature and those of Man. The optimum expression of synchronicity is found in wines that feature the qualities of the vine and territorial influences of the vintage (e.g., soil and climate). In terms of composition, we explain these distinctive organoleptic characteristics by examining a wine's aromatic and polyphenolic components, which are influenced by the vintage climate, vinification, and élevage in the winery.

Specifically for wine production, the link with the territory can be dictated by various factors that include the soil, the vine, the environment, and the use of specific transformation technologies, as they directly or indirectly interact with the native microflora. The yeasts are the main protagonists of the evolution from must to wine. Their presence in fermentation does not only result in a transformation of the sugars into alcohol, but also the formation of unique secondary products that add appreciable olfactory complexities to a wine.

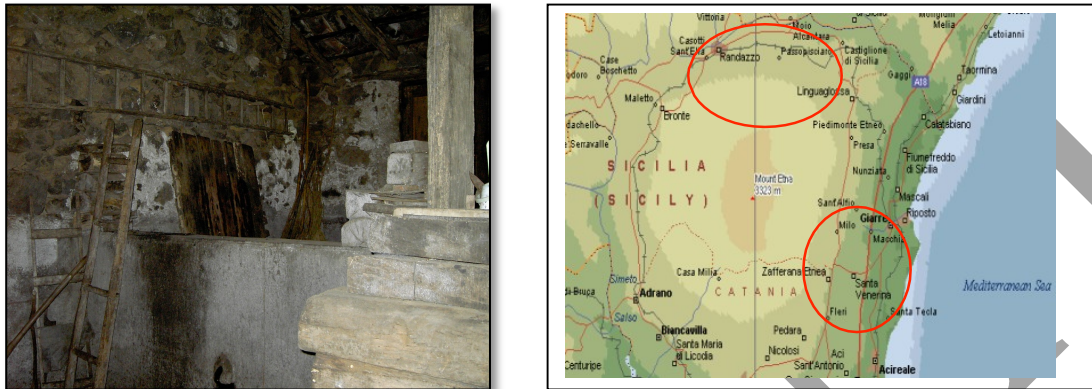
Our present work arises from the desire to protect the enological reality of the DOC Etna area by emphasizing the prestige of these wines, and focusing on their historic and territorial connections to the volcano Mount Etna.

The experimentation shown here was carried out by Benanti Winery, in collaboration with Sicily's *Regional Institute of Vine and Wine*. Our goals were aimed at isolating, studying, and characterizing yeast strains found during an exhaustive natural selection within the DOC Etna area. During the study, we also focused on each yeast's adaptation to the unique ecological microclimates, which revealed valuable peculiarities among the samples.

It is our belief that the associations between Mount Etna and the local traditions of wine production should not be standardized in a way that avoids or changes the true qualities of these wines. Every attempt must be made to nurture a permanent relationship between the indigenous grape cultivars and yeast strains found within the Etna DOC.

## Identification of Isolates

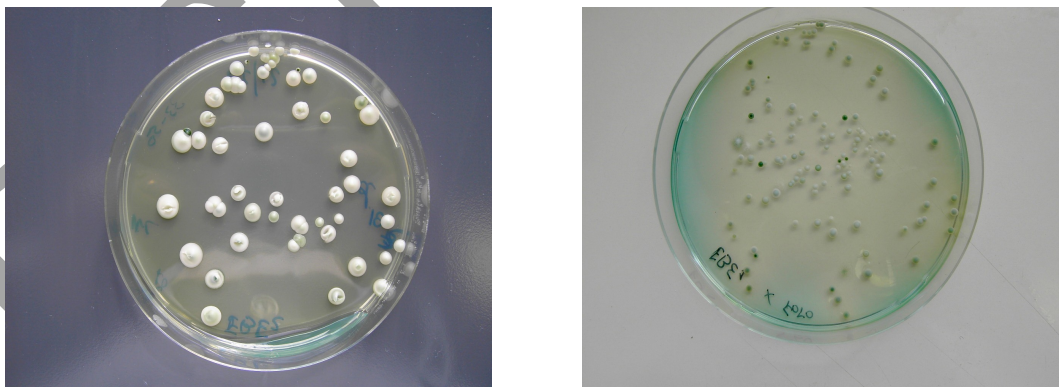
During the 2005 grape harvest, samples of must were taken directly from the fermentation vats in old palmenti at the end of alcoholic fermentation. The palmenti were located on small farms in the territory of the north and east slopes of the mountain, within the Etna DOC, where no commercial yeast strains have ever been used (*Fig. 1*).



*Fig. 1 — Ancient Etna palmento and areas of origin*

Using classical isolation techniques on selective substrates, 400 unique strains were catalogued during an initial collection (*Fig. 2*).<sup>1</sup>

Classical trials were conducted on isolated strains grown on specific media — *Yeast Extract Peptone Dextrose* (YEPD) and *Wallerstein Differential Agar* (WL). The presence of the genus *Saccharomyces* was confirmed by the inability to grow, after 5 days at 28°C on Oxoid Lysine Agar (*Fig. 3*). Subsequently, the species of *Saccharomyces* was determined by cultivating samples on a Vitamin-Free Yeast Base (*Fig. 4*).



*Fig. 2 — Yeast colonies isolated from musts at the end of alcoholic fermentation on WL*

(Cont'd)

<sup>1</sup> Cavazza and Poznanski 1998; Kurtzam & Fell, 1998

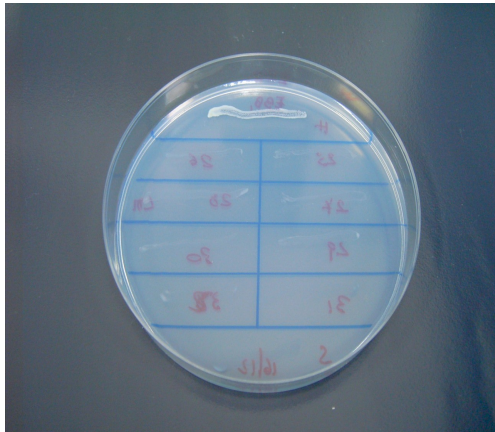


Fig. 3 — Plate with Agar-Lysine

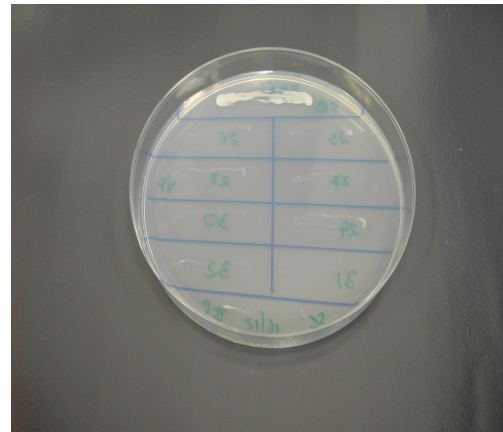


Fig. 4 — Vitamin-Free Plate

All samples of *Saccharomyces* identified were subjected to further analysis, including mitochondrial DNA polymorphism (RFLP) (Fig. 5).<sup>2</sup> Through this process we were able to reduce the collection to 160 unique strains of *Saccharomyces cerevisiae* (Tab. 1).

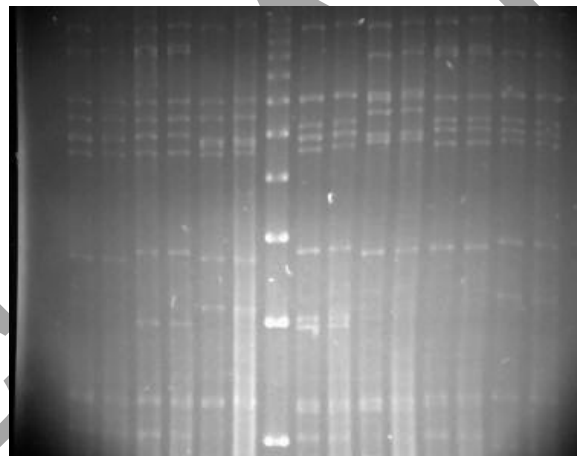


Fig. 5 — Agarose gel for the separation of mitochondrial DNA

Palmento	Location of Samples By Community	No. of Isolated <i>S. cerevisiae</i> Strains
B	MONTE LA GUARDIA	31
C	ROVITTELLO	45
E	MILO	53
F	SANTA VENERINA	31

Tab. 1 — *Saccharomyces cerevisiae* collection per palmento.

<sup>2</sup> All the samples identified were *Saccharomyces*. Those isolated in the initial phase were also other genera but were discarded because the purpose was to identify native *S. cerevisiae*.

## *Physiological And Technological Characterization of Isolated Yeasts*

The strains obtained from the first part of the work were subjected to a second phase of study which further defined the technological capabilities for each:

- good population development at low and high temperatures
- strong fermentations
- low residual sugar content after alcoholic fermentation
- tolerance to high ethanol production
- resistance to high levels of SO<sub>2</sub> and low production of the same
- low production of H<sub>2</sub>S
- low production of volatile acidity
- delicate foam formation

Although these parameters meet the basic requirements for a functioning oenological yeast, recent innovations have stimulated a broader understanding of yeast development and health, highlighting unexpected potential that, if fully realized, can obtain wines with genuine characteristics of unexpected freshness and purity. For example, the additional positive characteristics have been realized in the sample strains:

- good production of glycerine
- low production of acetaldehyde
- presence of specific enzymes such as  $\beta$ -glucosidase

At the end of this phase, thirteen potentially usable strains were identified as having ideal technological characteristics equal or superior to representative commercial yeasts used during other experiments in the winery.

During the 2007 harvest, all the strains underwent additional experimental microvinification trials of 100 liters using must obtained from Nerello Mascalese and Carricante grapes.

### *Vinification in White Wines*

Carricante grapes were harvested by hand and transported to the cellar in clean 18 kg boxes. The must obtained from a soft pressing of destemmed and unmacerated grapes was cooled to 5°C. After 24 hours, the clarified must was racked and allowed to rise in temperature to 18°C before being divided into steel tanks of about 100 liters each. Both experimental yeasts and commercial yeasts were added (at approx. 5% vol.) to initiate fermentation.

*(Cont'd)*

Fermentation took place at a controlled temperature of 18°C with an oscillation of 2°C. The commercial yeasts used for the checks were those commonly used in the Benanti winery, for their reliable technological and organoleptic qualities. Microbiological checks were carried out on samples taken from batches inoculated with yeasts throughout the fermentation, proved that the process was carried out entirely by the inoculated strains, without the influence of any other strains.

After primary (alcoholic) fermentation and malolactic conversion,<sup>3</sup> the wines were clarified using gravity and racked (to eliminate the lees) before being sulfited, filtered, bottled, and stored at optimum conditions. Ultimately, the wines were subjected to laboratory and sensory analysis, after aging for three and six months, and one year.

### ***Vinification in Red Wines***

The Nerello Mascalese grapes were harvested by hand and transported to the cellar in clean 18 kg boxes. The clusters were subjected to destemming (to remove the stalks) and the must (grapes, skins, and juice) obtained was divided into containers of about 100 liters each.

Inoculation occurred via selected yeasts and commercial yeasts (at approx. 5% vol.). The maceration and fermentation was carried out at 28°C for 11 days, with three gentle cap submersions per day. Frequent samples were analyzed to measure and record the microbiological changes in each trial, throughout the fermentation. The evidence confirmed that the process was carried out entirely by inoculated strains without the influence of other strains.

At the end of primary fermentation and maceration, the new wine was separated from each vat and the remaining skins were pressed.<sup>4</sup> The must was stored in steel containers, where the primary fermentation and malolactic conversion was completed.

The finished red wines underwent laboratory analysis, were sulfited, filtered, bottled, and stored in optimum conditions. Ultimately, the wines were subjected to laboratory and sensory analysis, after aging for three and six months, and one year.

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<sup>3</sup> Malolactic conversion (MLF) was done according to the vintage. If it was a cool year, partial to complete MLF is preferred. For warm vintages, MLF is typically partial.

<sup>4</sup> The press wines are kept separate from the free run (first flower).

## ***Primary Assessments***

Each lot inoculated with the cultivated Etna strains had a short lag phase and good fermentation kinetics, which finished slowly and evenly. In the must inoculated with the commercial strains, the kinetics were also fast and regular.

For both fermentations, foam production was minimal to absent. Laboratory analysis of the wines showed low production of acetaldehyde.

Organoleptic assessments carried out at the end of fermentation have highlighted positive olfactory and gustatory characteristics. Subsequent sensory analysis confirmed the uniqueness of each yeast used.

After evaluating each of the organoleptic characteristics, we focused our attention on four of the 13 strains, based on intensity, pleasure, persistence and above all the fineness of the final product, when compared against the same wine made with commercial strains.

During the 2008 harvest the four selected strains were used to inoculate experimental masses of 25 HL each. The same oenological practices used during the trials in 2007 were replicated. Foam production was low and the fermentation kinetics for all batches were fast and regular.

Chemical and sensory analysis confirmed our original assessments, showing strong similarities between the original microvinifications and the larger (25 HL) trials.

## ***Additional Organoleptic Details<sup>5</sup>***

The Carricante obtained with our selected yeasts produces a bright straw yellow color with an elegant ensemble of organoleptic characteristics.

In particular, we recognize the floral note of Etna broom (*ginestra*), yellow peach, sage, thyme and flint. The palate is savory, warm, substantial and balanced by a bright acidity and persistent freshness. (*Fig. 6*)

(*Cont'd*)

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<sup>5</sup> Translation key for analytical descriptors illustrated in Figures 6–8 (in clockwise direction, top to the right).

*Carricante (Fig. 6):* Straw yellow, florals, fruits, herbaceous, sweetness, acidity, body, bitterness, full flavor, persistence.

*Red wines (Fig: 7 & 8)* Ruby red, floral, fruity, color intensity, spicy, sweetness, acidity, body, bitterness, full flavor, persistence.

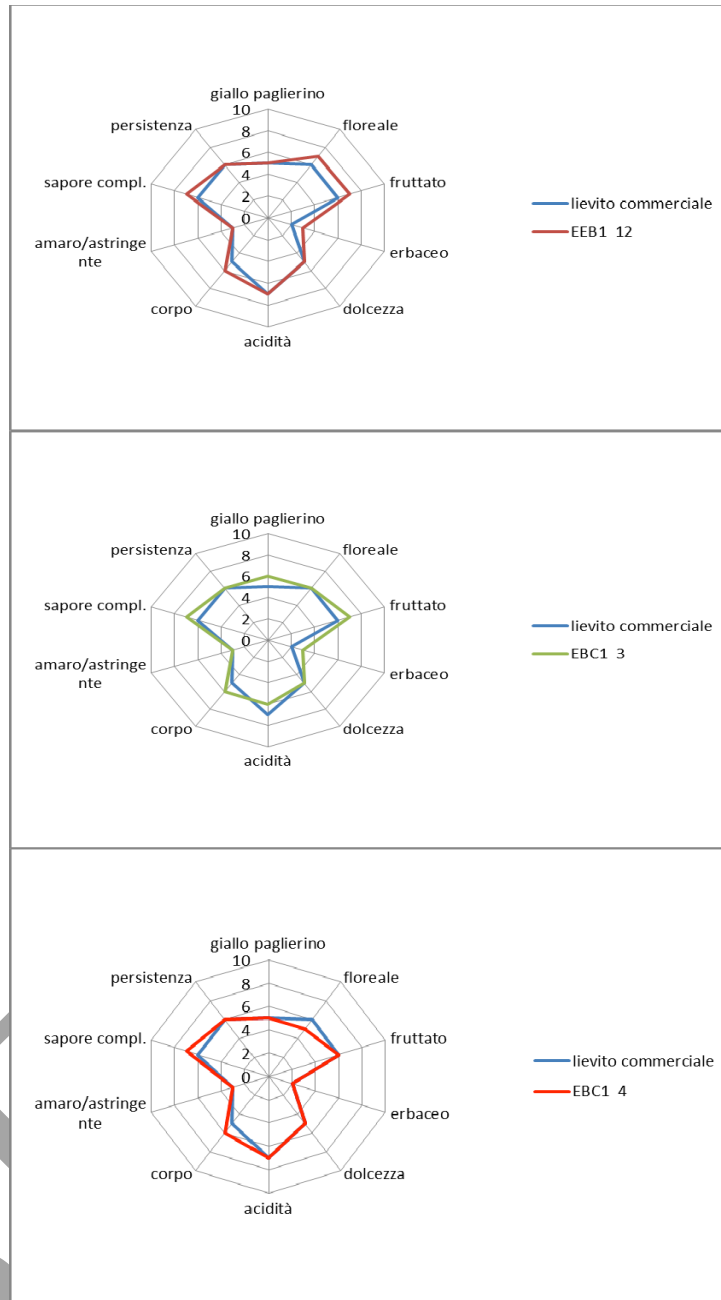


Fig. 6 — Aromatic profile of vinification 25 HI Carricante 2008

(Cont'd)



The same results were confirmed in the red wines. In particular, the Nerello Mascalese has a brilliant ruby red color with elegant and ethereal aromas of red fruit, forest floor and spice. The palate is well structured, with ripe and elegant tannins, and considerable persistence (Fig. 7).

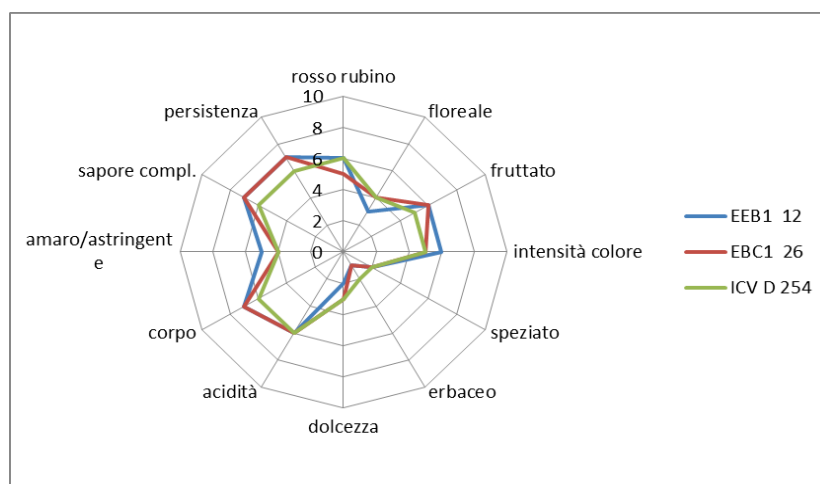


Fig. 7 — Profile of aromatic vinifications 2008 Nerello Mascalese 25 HL

In Nerello Cappuccio the deep ruby red color leans toward purple. On the nose, the prevailing aromas are of ripe red fruit, with sweet fruit flavors and a supple structure, a moderate persistence and broad satin tannins (Fig. 8).

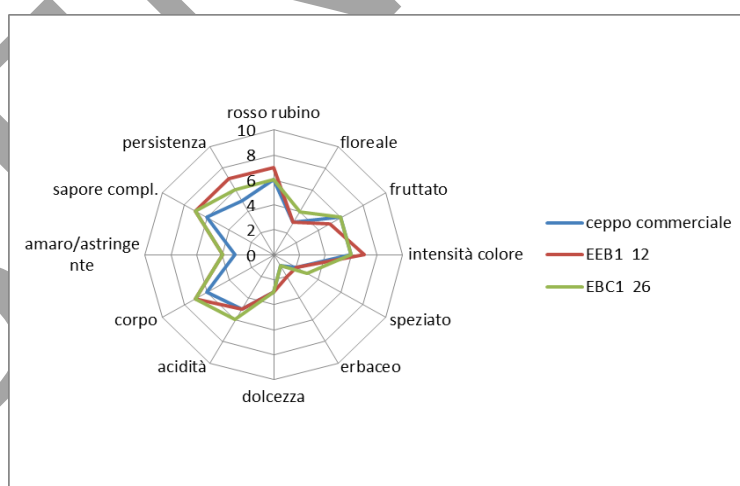


Fig. 8 — Aromatic profile of vinification 2008 Nerello Cappuccio 25 HL

(Cont'd)

Tab. 2 — Patented yeast characters.

For the strains that gave the best results, a patent was requested and obtained

Strain	EBB <sub>1</sub> 12	EBC <sub>1</sub> 3	EBC <sub>1</sub> 4	EBC <sub>1</sub> 26
<b>CHARACTERISTICS OF STRAINS</b>				
Fermentation of Sucrose	+	+	+	+
Fermentation of Maltose	+	+	+	+
Assimilation of Galactose	+		+	-
Assimilation of Trehalose	-	-	+	-
Assimilation of Raffinose	+	+	+	-
Starch Assimilation	-	-	-	-
<b>UNIQUE CHARACTERISTICS</b>				
Color	Chartreuse	Chartreuse	Chartreuse	Chartreuse
Rise	Relief	Relief	Relief	Relief
Surface	Smooth	Smooth	Smooth	Smooth
Consistency	Creamy	Creamy	Creamy	Creamy
Development	Powdery	Powdery	Fluffy	Powdery
Development of Lysine	Absent	Absent	Absent	Absent
Accumulation of Vitamins	Absent	Absent	Absent	Absent
Fermentation of Glucose	+	+	+	+
Assimilation of Cellobiose	-	-	-	-
Assimilation of Citrate	-	-	-	-
Assimilation of Nitrate	-	-	-	-
Growth at 30 °C	+	+	+	+
Prod. of Cycloheximide 0.01%	-	-	-	-
<b>ENOLOGICAL CHARACTERISTICS</b>				
Ethanol Toxicity	Resistant	Resistant	Resistant	Resistant
Alcohol Production	16-18 % V/V	16-18 % V/V	13-15 % V/V	16-18 % V/V
Production of Volatile Acidity	Low	Low	Low	Low
Speed of Fermentation	Rapid, Regular	Rapid, Regular	Rapid, Regular	Rapid, Regular
Resistance to Sulfur	Good	High	Elevated	High
Temperature Range	12-38 °C	14-35 °C	13-32 °C	14-35 °C
Sedimentation capacity	High	Elevated	High	High
Foaming Potential	Low	Low	Low	Low
Production of Tartaric Acid	Low	Low	Low	Low
Production of SO <sub>2</sub>	Low	Low	Low	Low

## *Conclusions & Patents*

Several studies have shown that the use of starter yeasts can reduce the production of “undesirable” metabolic components (e.g. high alcohols; isoamylacetate; ethyl acetate, etc.), which are detectable in wines fermented spontaneously by yeast strains (*microbiota*) found in the immediate environment.

It is also true that the ideal of “a perfect fermentation ” has changed over time. As a result, the rationality of the fermentation process has matured to a point where we also seek balance and maximum expression of natural factors in each fermentation.

The choice of starter, according to its particular metabolic characteristics, is a strategic one. Each yeast influences the typicity of a must through lysis—the transfer of discernable organoleptic qualities at a cellular level—which can modify a wine’s structure and the arrangement of volatile compounds.

Inoculation with selected yeasts is one of many well-established practices designed to “control” the final composition of a wine, eliminating the randomness factor often found in spontaneous fermentations.

The problem with many selected yeasts currently in the market is that the strains used to create them are isolated from distant ecosystems, which differ significantly from those where they are often applied. The pre-packaged yeasts perform the fermentation process, but they do not fully exploit the territorial characteristics the way that indigenous yeasts can.

This is why we have decided to patent the yeast strains discovered during our research, for use exclusively in our winery. The indigenous yeasts discovered during these trials have adapted to local grape varieties and terroir of Mount Etna. By implementing the use of cultivated indigenous yeasts, which have adapted to the environs and grape varieties of the volcano, we amplify and celebrate the territorial connections in every Benanti wine.

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