Sequential inoculation with non-Saccharomyces yeast offers new possibilities for boosting sensory quality.

Lallemand is exploring the use of non-Saccharomyces with aromatic properties that are unique from those found in Saccharomyces, in order to contribute more complex aromatic profiles while also ensuring fermentation security.
**BIODIVERSITY,**

Biological diversity is defined as the diversity of all life forms. From a scientific perspective, that means all varieties of life forms are based on three levels:

**Environmental diversity:**
Berries, soil, vine leaves, tank, viticultural and winemaking practices...

**Species diversity:**
Saccharomyces, Metschnikowia, Cryptococcus, Hanseniaspora, Kloeckera, Pichia, Torulaspora, Brettanomyces...

**Diversity of individual elements within each species:**
Lallemand offers more than 150 strains of Saccharomyces for winemaking. Interactions among yeast species can be monitored during spontaneous fermentation by studying the dynamics of the different populations.

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**BIODIVERSITY BACKGROUND**

1870
Louis Pasteur’s work leads to a better understanding of the fermentation process.

1970
Active Dry Yeasts are used regularly to control alcoholic fermentations.

1985
Over 150 strains of Saccharomyces cerevisiae are selected and available for controlling alcoholic fermentation.

2000
New technologies reveal unique yeast species.

21st century
With the appearance of biodiversity in the pre-fermentation phase, new possibilities open for modern winemaking. A revolution in the field of microbiology that offers a greater sensory universe.

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In your opinion:
Are all yeast strains of Saccharomyces cerevisiae compatible in sequential inoculation with other:

1 Saccharomyces cerevisiae yeast?
2 Torulaspora delbrueckii yeast?
3 Metschnikowia pulcherrima yeast?

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Scientists have observed that non-Saccharomyces yeasts are particularly active during the pre-fermentation phase. This under-exploited period allows the non-Saccharomyces yeasts to reveal precursors and aromatic compounds in must.

Details of the principal microbial phases of vinification:

1. **Pre-fermentation phase**
   - The “non-Saccharomyces” yeasts generally dominate the grape must and produce precursors and aromatic compounds during this phase. This phase can be dangerous for alcoholic fermentation if these yeasts deplete the medium of its nutrients and produce large quantities of compounds that are undesirable for varietal and “terroir” expression.

2. **Fermentation phase**
   - Here Saccharomyces is dominant as it is the best-adapted species for using sugars, at the same time it also produces fermentation aromas (largely derived from precursors that are present in the must).

3. **Maturation phase**
   - Where the spoilage apiculate yeasts look for opportunities to grow.

**Fermentation solutions**
Lallemand, the specialist in research and production of biological, offers a new generation of biodiversity-related yeasts: Level² Solutions. Innovative solutions pairing Saccharomyces with non-Saccharomyces species harnessing biodiversity.

**The decision to focus on sequential inoculation**
Lallemand has demonstrated that sequential inoculation results in optimizing the contributions of biological diversity.

**The world of oenology is on the move, Lallemand recommends tomorrow’s solutions.**
A new approach to vinification has opened the way for modern oenology, exploiting the best that nature offers and retaining the intrinsic character of the wine.
Enhance aromatic complexity and mouthfeel

Originally released in kit form as Level2 TD™ for white wines, Lallemand has relaunched this yeast as a single product - BIODIVA™. Now winemakers can select *Torulaspora delbrueckii* and pair it with the appropriate *Saccharomyces cerevisiae* strain to contribute to mouthfeel and an overall increase in aromatic complexity. Suggested varieties include Chardonnay, Sémillon, Syrah and Pinot Noir as well as fermenting late harvest and ice wines.

*See a Lallemand representative to discuss a suitably paired *Saccharomyces cerevisiae* yeast.

Over-express aromatic compounds in your white and rosé wines

FLAVIA™ is a pure culture of *Metschnikowia pulcherrima* selected from nature by the Universidad de Santiago de Chile (USACH) for its specific capacity to release enzymes with α-L-arabinofuranosidase activity. When used in sequential inoculation with a compatible selected *Saccharomyces cerevisiae* yeast strain, FLAVIA™ will impact the production of such varietal aromas as terpenes and volatile thiols. Under certain conditions, *Metschnikowia pulcherrima* does not show fermentative activity. However, the enzymatic activity of *Metschnikowia pulcherrima* gives the desired outcomes. Best results are achieved when FLAVIA™ is sequentially inoculated with a *Saccharomyces cerevisiae* strain that is a high terpene/thiol releaser/converter. Suggested varieties include Riesling, Sauvignon Blanc and Colombard.

*See a Lallemand representative to discuss a suitably paired *Saccharomyces cerevisiae* yeast.

**Comparative trial on Syrah 2011 (Rhône Valley): impact of Biodiva™ on the sensory profile**

*Blind tasting, 27 tasters*

**White Wine (Colombard Grape variety) from South of France, Vintage 2011. Sensory Analysis by 14 wine experts 1 month after bottling.*
The range of new opportunities

This is just the beginning of an entire array of opportunities that will pave the way for even better control of fermentation, using natural yeasts that have been selected for winemaking.

With Level² Solutions, Lallemand continues to pioneer the introduction of new species and new methods for managing alcoholic fermentation with reliable solutions that will open new winemaking opportunities.

The wide variety of selected natural yeasts reflects the biodiversity, and yet this diversity is still underexploited despite the large number of species and subspecies (other than *Saccharomyces cerevisiae*) that are present in most grape musts. During spontaneous fermentation, actual microbial population dynamics result in successions of enzyme activity that undoubtedly contribute, positively or negatively, to the aromatic complexity and diversity of the wine. Thanks to Lallemand R&D research program, the management of Alcoholic Fermentation introducing the use of non-conventional selected yeasts such as *Torulaspora delbrueckii* and *Metschnikowia pulcherrima* in sequential inoculation with *Saccharomyces cerevisiae* opens new possibilities for winemakers.

After years of R&D, Lallemand is proud to produce two non-*Saccharomyces* yeast strains - *Metschnikowia pulcherrima* and *Torulaspora delbrueckii* - for use in sequential inoculation with a paired *Saccharomyces cerevisiae* strain. The sensory contribution of the non-conventional yeast in conjunction with the security of the *Saccharomyces cerevisiae* enable winemakers to impact the sensory qualities and complexity of their wine while ensuring a reliable and complete fermentation.

Different production strategies of non-*Saccharomyces* in the form of active dry yeast (ADY) were implemented to increase survival rates in difficult enological conditions, and to increase their capacity to multiply and establish themselves in grape musts.

With the improvements in our ADY production processes, we are now able to produce non-*Saccharomyces* yeasts that meet sustainability and vitality as reliably as *Saccharomyces cerevisiae* ADY.
Glossary

- **Sequential Inoculation**: Successive inoculations of two selected micro-organism populations
- **Selected Active Dry Yeast**: Selected wine yeast, multiplied and dried for alcoholic fermentation management
- **Micro-organisms populations dynamics**: Evolution and interaction over time of different micro-organism populations present in the must during fermentation
- **Alcoholic fermentation**: Transformation of sugar into alcohol, mainly due to the metabolism of *Saccharomyces*
- **Pre-fermentation phase**: Phase during which many micro-organisms impact the quality of the alcoholic fermentation, and hence the wine sensory properties
- **Fermentation aromas**: Metabolism-related yeast aromas during alcoholic fermentation
- **Aromatic precursors**: Compounds converted by yeast metabolism to aromatic compounds
- **Indigenous microflora**: Micro-organisms present on the grape and winery environment
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