Fermentations management: tools for the preservation of the wine specificity.

La gestion des fermentations, des outils pour la révélation et la préservation de la typicité des vins.

Vincent RENOUF[1,3*], Charlotte Gourraud[1] and Marie-Laure Murat[2]

[1]Laffort, BP 17, 33072 Bordeaux cedex 15, FRANCE.
[2]Laboratoire SARCO, filiale de recherche de la société Laffort, BP 40, 33072 Bordeaux, FRANCE.
[3]ENITA de Bordeaux, 1 cours du général de Gaulle, 33175 Gradignan cedex, FRANCE.

***Corresponding author:** vincent.renouf@laffort.com

Abstract

Development of the indigenous microflora is not insignificant on the wine quality. *S. cerevisiae* indigenous strains are low tolerant to ethanol. They can lead to sluggish fermentations. *B. bruxellensis* produce volatile phenols affecting fruity and freshness wines characters. Some indigenous *O. oeni* strains can be responsible for the presence of biogenic amines in wines. To overcome these problems, the use of selected yeast and bacteria strains is the most efficient tool. However, controlling the native flora industrial strains should not reduce the singularity of each wine.
Selection process should take into account the aromatic profile of the strains in addition to their fermentative capacity. Researchers should provide large pool of strains suitable to be used for different types of wines. These considerations are crucial for white wines where it is essential to encourage the expression of the varietals qualities. In red wines, strain neutrality aromatic is more recommended and the focus should be made on fermentative kinetics and microbial security.
The objective of our work is to raise question of the specificity and the diversity of the microbial species and strains involved in winemaking. Probably stemming from their isolation origin, strains exhibited several differences which should be used to encourage the preservation of the differences between each type of wines and to respect the 'terroir' impact and the originality of each wine.

Key words: microbial diversity, yeast, bacteria, strains, wine typicity, spoilage.

Introduction: the diversity of the yeast and the bacteria involved in winemaking

The microbiological part in the concept of 'Terroir' is the source of several debates. Whatever the case, the natural presence of wine micro-organisms at the vineyard is now recognized. Indigenous yeast strains of *Saccharomyces cerevisiae* and *Brettanomyces bruxellensis* as well as the bacteria *Oenoccocus oeni* are isolated on the berries surface. They are different according to the vineyard (Renouf et al. 2006). The causes and consequences of this observation are not yet fully apprehended.
At the vineyard forty or fifty yeast and bacteria species are currently detected. The phylogenetic tree presented in the figure 1 and similar results obtained for yeast illustrate the great diversity and the complexity of the microbial consortium of the berries surface (Renouf 2006). Theses indigenous species can persist in must after berries crushing, and also in wine during the first stages of the alcoholic fermentation as it is evocated in table 1.

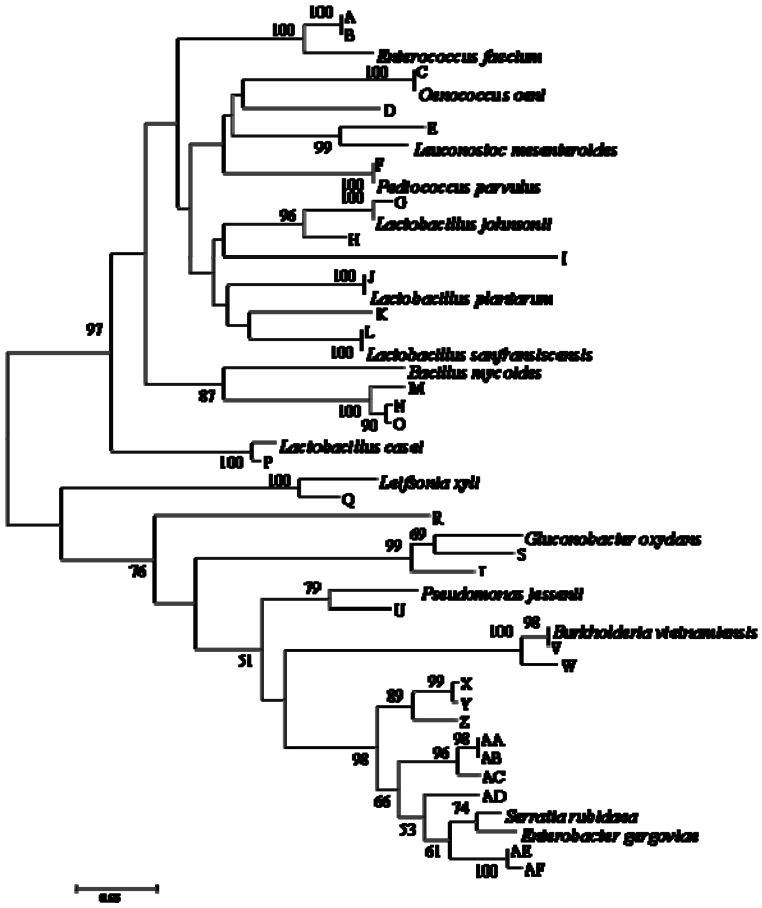

Figure 1 Phylogenetic tree built according to the neighbour-joining method with bacteria sequences detected from grape surface analysis.

			Density= 1.08	Density=1.0
Yeast	Non-fermentatitive species	*Rhodotorula graminis*	×	-
		Bulleromyces albus	×	-
		Sporobolomyces roseus	×	-
	Low Fermentative species	*Metschnikowia fructicola*	×	×
		Candida stellata	×	-
		Pichia anomala	×	×
	Fermentative species	*Saccharomyces cerevisiae*	×	×
		Brettanomyces bruxellensis	×	×
Bacteria	Lactic acid bacteria	*Oenococcus oeni*	×	×
		Lactobacillus plantarum	×	×
		Pediococcus parvulus	×	×
	Acetic acid bacteria	*Gluconobacter oxydans*	×	-
		Acetobacter aceti	×	×
	Other bacteria	*Enterococcus sp.*	×	-
		Bacillus sp.	×	-

Table 1 Yeast and bacteria species identified in must during indigenous AF for Merlot wine.

The indigenous species can have different impact on wine sensorial qualities; some are prejudicial to the wine quality. Among the bacteria, *Pediococcus* and *Lactobacillus* species can degrade higher amounts of glycerol than *Oenococcus oeni* for instance (Claisse et al. 2001). Concerning the yeast, *Pichia* and *Candida* species produce large amounts of ethyl acetate whereas *Saccharomyces* generates small amounts of this ester but higher quantities of benefit fermentative esters (isoamyl acetate…) (Plata et al. 2003). Certain metabolisms are prejudicial to the wine typicity; it is the case of the volatiles phenol by the yeast *B. bruxellensis* (Chatonnet et al. 1992).

How manage yeast and bacteria in order to preserve wine typicity and diversity?

Control the development of the undesirable species.

The vineyard origin of *B. bruxellensis* has been recently highlighted (Renouf and Lonvaud-Funel 2007). In must, *B. bruxellensis* is still latent. But if *S. cerevisiae* cells decline before the end of the total sugar consumption, *B. bruxellensis* can take benefit to grow in wine. That can explain apparition of phenolic off-flavour during sluggish AF. To prevent this alteration, use of efficient selected strains of *S. cerevisiae* associated with a management of its nutrition by addition of activator, supplementation in nitrogen and vitamins are the best method.

Then MLF is a key stage for *B. bruxellensis* development in wine. The natural progression of indigenous fermentations leaves a microbiological void between yeast decline and the bacteria development. This microbiological void encourages the development of *B. bruxellensis*. An efficient inoculation of an active *O. oeni* strain reduces the time required to begin MLF. Thus, *B. bruxellensis* cannot develop as the microbial ecosystem is never in void status (Renouf and Murat 2008).

In addition, MLF starters have a better fermentation capacity than indigenous strains. MLF performed with malolactic starters are faster than indigenous fermentation, post-fermentative SO_2 addition, which is a crucial action inhibiting *B. bruxellensis* and other indigenous spoilage species, is anticipated. Residual *B. bruxellensis* and *O. oeni* populations after SO_2 addition are significantly less important in the wine fermented with the malolactic starter (table 2). Therefore, risks of deviation are less important and the stabilization of the microbial ecosystem during ageing is anticipated.

	Indigenous Microflora	Malolactic starter (Lactoenos 450preAc®)
B. bruxellensis population (CFU/mL)	6.3×10^3	2.10^1
O. oeni population (CFU/mL)	3.5×10^4	<1

Table 2 Effect of inoculation with selected lactic bacteria (Lactoenos 450 preAc® by LAFFORT) on the wine's post-MLF (30 d after SO2 addition) microbial populations.

Starters additions are the better tools to prevent the deviations resulting from indigenous microflora during winemaking. Nevertheless, management of the native flora by the use of industrial strains should not reduce the singularity of each wine, which may be the consequence of the natural microbial diversity lost by the use of the similar fermentative strains for different types of wines.

Use of the strain diversity, How to benefit from the diversity between strains?

Since the recognition of their usefulness, development of effective *S. cerevisiae* strains for the AF and *O. oeni* strains for the MLF are the focus of lots studies. Criteria taken into account are the intrinsic capacity to survive and to grow in wine and the fermentation ability. To provide yeast and bacteria in a range broad increasingly and to respect the variations of each wine, other tests were added to these phenotypic basic criteria. It is the case of high production test for positive metabolites and on the contrary the low production of undesirable metabolites.

The most famous case of yeast impact on concerns yeasts in white wines. Indeed, *S. cerevisiae* yeast species is the leading actress revealing the aromatic potential of white wine varietals notably for the Sauvignon *blanc*. The objective is to favour the release of aromatic compounds from the odourless grape precursors. It is the case of the volatile thiols: the 4-mercapto-4-methylpentan-2-one (4MMP), the 3-mercaptohexan-1-ol (3MH), and the 4-mercapto-4-methylpentan-2-ol (4MMPOH). The production ability is directly dependent on the strain. Important differences can be observed like it is illustrated by the figure 2 (Murat et al. 2000). Volatile thiols are not the only compounds produce by the *S. cerevisiae* strain contributing to the wine complexity. It is also the case of the esters.

		Zymaflore X16® by Laffort (France)	Zymaflore X5® by Laffort (France)	Zymaflore VL3® by Laffort (France)
Volatile Thiols (ng/L)	4MMP	32,5	97,5	36,3
	3MHA	28	46,8	33
Fermentative Esters (mg/L)	Isoamyl acetate	4,6	2,1	1,8
	Phenylethyl acetate	2,7	1,6	2,3

Table 3 Comparison of three S. cerevisiae strains (Sauvignon blanc)

The Zymaflore X5®'s volatile thiols production is the highest. But its esters production is lowest. About the Zymaflore X16® strain his esters production is relatively important and its volatile thiols are less high. Zymaflore VL3® presents a medium production of volatile thiols but the lowest esters production. Here it is not the esters but the varietals aromas in a Sauvignon *blanc* which is largely encouraged.

In red wines, fermentative esters have a great importance to the sensorial wine properties. They participate to the wine's freshness and to its fruitiness. Therefore, the strains with high ability to produce theses esters can be favoured. By comparison with Sauvignon previously cited, for Syrah wine favouring a strain with important intrinsic ability to produce volatile thiols is not appropriate and it is more interesting to produce esters.

		Zymaflore Rx60®
Volatile Thiols (ng/L)	4MMP	< detection threshold
	3MHA	< detection threshold
Fermentative Esters (mg/L)	Isoamyl acetate	9,5
	Phenylethyl acetate	80

Table 4 Fermentative esters and volatile thiols concentration in a Syrah wine at the end of the AF performed with the Zymaflore Rx60® strain provided by LAFFORT (France).

In red wines, like for white wines, productions of undesirable metabolites should also take into account during the selection of the strain. In red wines, it concerns notably the ability to produce vinyl phenols, which can confers synthetic and medicinal off odours to the wine and also serve to substrates for *B. bruxellensis* to produce spicy and animal depreciating characters. In white wines, that can be the ethyl acetate notably in sweet wines leading vinegar smell. Theses productions are also strain dependant as it is reported in the Table 5.

		4-vinylphenol (µg/L)	4-vinylgaïacol (µg/L)
Cabernet-Sauvignon	Strain A	51±4	4,1±0,5
	Strain B	116±8	9±1
	Reference Strain (522 Davis)	214±14	16±2
		Ethyl acetate (mg/L)	
Semillon	Strain C	155±12	
	Strain D	62±7	
	Reference Strain (522 Davis)	112±4	

Table 5 Differences between the S. cerevisiae strains: For the red wine (Cabernet-Sauvignon): production of 4-vinylphenol and 4-vinylgaïacol. For the white wine (Semillon): production of ethyl acetate.

In the two cases, production of the compounds altering varies as much as three times. For the less productive strain the concentration is still below the detection threshold, but for the highest productive strain we have reached detection threshold, alteration is noted by the tasters. For the Semillon wine, the excessive production of ethyl acetate of the strain C reveals an ascescence characters detrimental to fruity, freshness, and floral note by comparison with the tasting of the less productive strain (Figure 2).

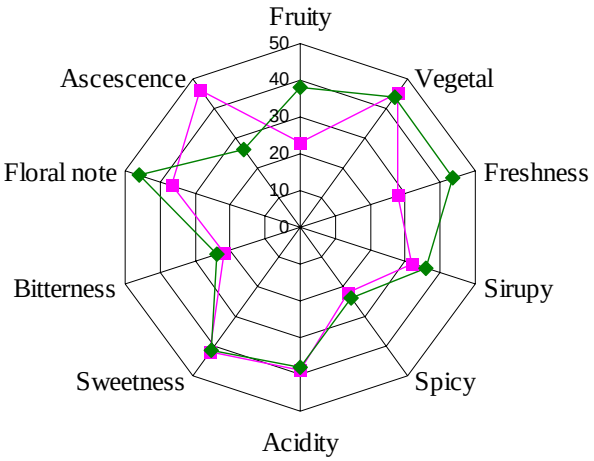

Figure 2 Diagram of comparative tasting of the B (with pink squares) and C (with green diamond) strains for the Semillon wine

Lactic acid bacteria have also their impact on the wine sensorial properties. They contribute to a loss of acidity. Greenness character conferred by the L-malic acid is reduced by the sweet and sour note of the L-lactic ac id. The LAB strains also produce diacetyle (Batowsky and Henschke 2004) which confers buttery notes. In fact diacetyl at low concentration will impact nutty and toasty aroma whereas at high concentration, it has a characteristic buttery aroma that is associated with a lactic character for which the appreciation varies according to tasters. In all cases exceeding 5 – 7 mg/L diacetyl is regarded by many tasters to be a spoilage character (Davis et al. 1986). Concentrations of diacetyl in wine depend on the ability its production of strains conducted for the MLF. When MLF is carried out in oak wood barrels or with oak chips, LAB are also able to interact with wood and increase volatile compound content such as vanillin or whiskylactone. Here also, this ability is strain dependant (Bloem et al. 2008).

Like for the yeast, all bacterial metabolisms are note beneficial to the wine. Some metabolites produce by the LAB altering the wine. It is also the case of the 4-vinylphenol and the 4-vinylguaiacol. Like for *S. cerevisaie* strains, some LAB species are able to produce these compounds. Natural species currently detected during indigenous fermentation can produce theses compounds whereas the *O. oeni* species which is used for the production of malolactic starter presents very low ability of prodcution(Table 6). It is also important to note that some indigenous LAB species, *Lactobacillus plantarum* and *Pediococcus damnosus* are also able to convert the 4-vinylphenol and the 4-vinylguaiacol into the 4-ethylphenol and the 4-ethylguaiacol (Table 6) (Couto et al. 2006). That can amplify the *B. bruxellensis* activity leading to spicy and animal off-odours strongly depreciated by the consumers because that reduces the freshness and the typicity of the wine.

	4-vinylguaiacol (µg/L)	4-vinylphenol (µg/L)	4-ethylguaiacol (µg/L)	4-ethylphenol (µg/L)
O. oeni	24±4	42±2	0	0
Lb. plantarum	4±1	132±24	8±2	88±12
Pd. damnosus	44±2	32±2	16±2	22±2

Table 6 Volatile phenols production by different indigenous LAB species isolated from Merlot wines.

Biogenic amines production affects the consumers heath is the case of the biogenic amines (Landete et al. 2007). It is also strain dependent. The absence of this metabolism is a criteria taken into account to the selection of malolactic starter. When an efficient malolactic starter is used the concentration of histamine are always less important than in an indigenous fermentation (Table 7).

	Indigenous microflora	Lactoenos SB3®
Histamine (mg/L)	44±4	< detection threshold

Table 7 Production of histamine at the of MLF conducted with indigenous microflora and with Lactoenos SB3® strain (by LAFFORT, France).

Conclusions

Lots species of yeast and bacteria are suitable to act during the transformation of the grape juice into the wine. Some are beneficial and contribute to reveal the qualities of the grapes, while others are harmful. Monopolized the ecosystem by selected strains is the best tool to frame the growth of undesirable indigenous strains. However, the use of yeast and bacteria starters should not lead to homogenisation of the contribution of micro-organisms to the wine qualities. We must control the species alteration while exploiting the diversity that exists naturally between strains of positive species. That leads to introduce the concept of biodiversity in wine. In order to offer the most diverse strain possible fitting different objectives products, it is crucial to examine their differences and their impact on the wine quality. The activities beneficial in terms of revelation varietals compounds are very interesting since they perfectly suited to the development of wine well typical. While the general metabolites can stick targets to different products for which fermentation aromas are appreciated. Finally, during strains selection, lack of metabolic alteration should obviously be controlled. Further works should be continued in order to better understand the phenomena that lead to the diversity between strains and how to use them to develop strains of increasingly powerful and diverse as possible

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