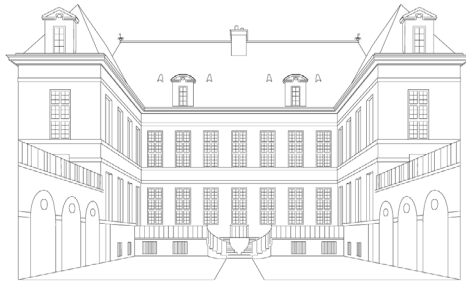




# USE OF BIOPROTECTION STRAINS IN WINEMAKING



# *OIV* 100

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## **BACKGROUND**

The Microbiology expert group of the OIV has chosen to undertake this review to bring together the knowledge that Member States and Observers have on the bioprotection in winemaking. This review does not attempt to cover in detail all the issues and facts, but rather to outline the applications of bioprotection by highlighting some of the current information and techniques available for wine production.

## **SCOPE**

The purpose of this document is to assemble the main important elements of bioprotection in winemaking and some of the recent studies conducted on this topic in a single document.

## INTRODUCTION

Bioprotection is a tool that is developing quickly. It must be considered that this draft is done with the current information and many things will be discovered in the near future, especially concerning conditions of use: compatibility between yeasts, with several strains of *Saccharomyces*, between *Saccharomyces* and non-*Saccharomyces* and when using yeasts/bacteria associations. Many aspects must be assessed in terms of nutritional needs and fermentation conditions (e.g. temperature, sulfites). The effectiveness of bioprotection will depend on the parameters in the wine matrix and the initial wild population of yeast and bacteria can influence this. The use of different strains for fermentation and bioprotection can pose implementation problems, such as the timing of inoculation. Some non-*Saccharomyces* yeasts are now used for fermentation, and they could have a double fermentative/bioprotective effect. It is difficult to classify the use of yeasts and lactic acid bacteria (LAB) for bioprotection or just for fermentation.

The main applications of bioprotection are focused to limit the undesired initial indigenous populations that can be detrimental to fermentation objectives and to control oxidation. The latter aspect is not completely clear and further research is necessary to support it. Bioprotection should include these objectives, but it is difficult to separate this role from the biotransformation of the matrix (grape/juice) by fermentation. The effectiveness or performance in bioprotection of a specific microorganism (yeast or LAB) is difficult to measure therefore evaluation of its role is complex. There are no clear parameters of individual microorganisms, except to measure the overall wine parameters during fermentation.

The use of LAB can be considered as a potential way of bioprotection for the early control of *Brettanomyces*, as some recent evidence supports, but further research must be done to clarify the conditions of the application and how to separate the fermentative role from the bioprotective effect. The role of some strains of *Lactobacillus plantarum* towards acetic bacteria was also shown in wine.

The following recommendations can be applied to grapes with a suitable sanitary state and maturity. Some preliminary evidence suggests that grapes harvested at a later stage could require higher doses of bioprotection agents.



## DEFINITION OF BIOPROTECTION

Use of oenological microorganisms, by direct effect or through some derivatives (zymocins, bacteriocins...) produced by the inoculated protective microorganisms and not added as purified products, to control the development of other undesirable microorganisms and/or to avoid oxidations, to reduce SO<sub>2</sub> use in grapes and wines and to preserve the sensory properties of the final product. Bioprotection can be considered as a full or partial alternative to other chemical products used to control oxidations or microbial developments and for:

- Protection of against undesirable microbial populations,
- Protection of the wine matrix,
- Protection of the final product in mind.

**Scope/Object:** Control unwanted microbial development and/or oxidation on grape, must and wine.

**Microorganisms used:** Selected yeasts (*Saccharomyces*/*non-Saccharomyces*), LAB (*O. oeni*/*L. plantarum*...), and microbial derivatives (zymocins, bacteriocins...) produced by the inoculated protective microorganisms (during the actual fermentation) and not added as purified products. As some strains can show higher effectivity than others the strain-effect must be considered between bioprotective species.

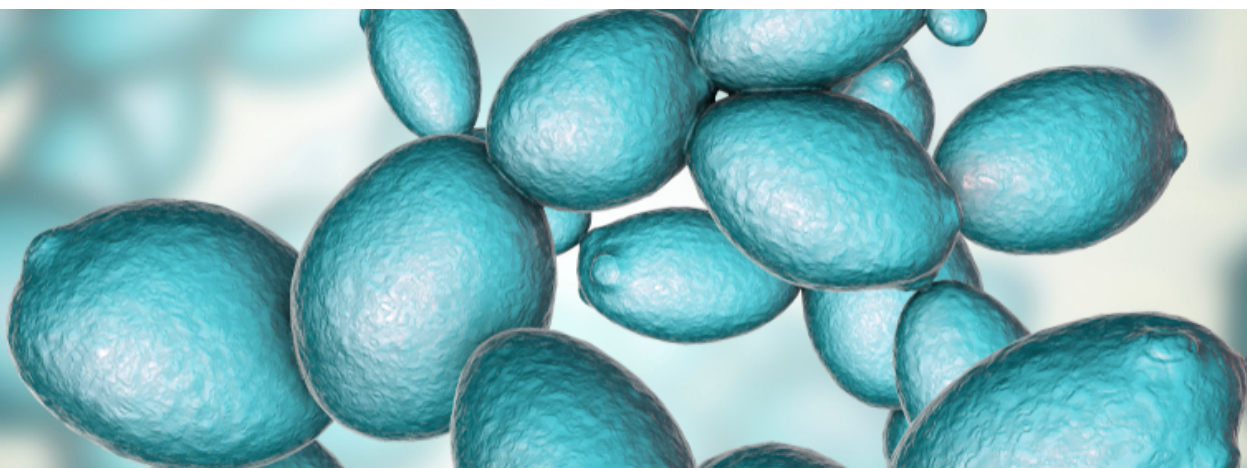
**Timing and application:** On grapes, pre-fermentative at machine harvesting, transport, during crushing, press, settling, during alcoholic fermentation or post fermentation.

**Temperature:** In the case of white and rosé winemaking it is advised to keep the must at lower temperatures during clarification to obtain better results, depending on the bioprotection strains used without falling below the minimum temperature for their reactivation.

**Initial population:** Bioprotection is a powerful tool but with some limitations. If the initial populations on grapes or in must is very high the effectivity can be low. So, bioprotection must be applied on healthy grapes with normal levels of wild microorganisms (<10<sup>4</sup> CFU/mL for yeasts, <10<sup>2</sup> for bacteria). In terms of niche occupation, bioprotection is less effective at advanced maturity, although still possible.

**SO<sub>2</sub> resistance:** Some strains are only resistant to low levels of SO<sub>2</sub> (max. 30 mg/l). Bioprotection and SO<sub>2</sub> can be combined, but care must be taken not to exceed the dose limit.

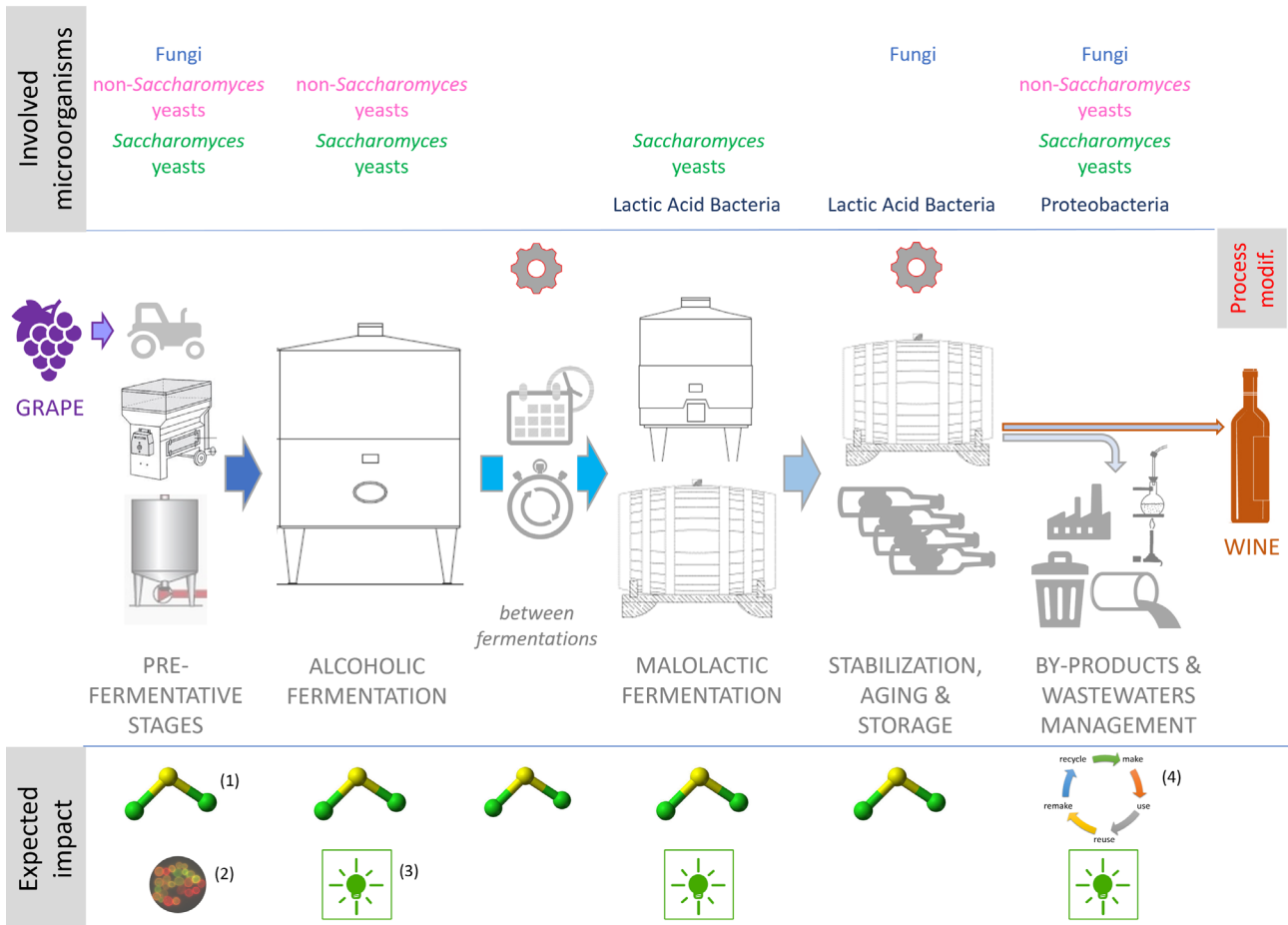
**Dose:** A minimum dose of 5 g/hL is recommended, when yeasts are applied pre-fermentation, but the inoculum can be adapted depending on the species, initial indigenous population, grape/must composition, maturity level, pH and temperature. While using commercial strains, it is recommended to follow the supplier recommendations.







**Fig A1.** From: Nardi, T. Microbial Resources as a Tool for Enhancing Sustainability in Winemaking. *Microorganisms* 2020, 8, 507. <https://doi.org/10.3390/microorganisms8040507>



(1) Sulfite reduction (2) Biodiversity improvement (3) Energy savings (4) Reuse and valorization of by-products.



**Table 1.** Some potential applications of bioprotection in grapes and musts. To be considered as examples and can be completed or improved with other applications. Noted that not all the species in the list are commercially available.

Yeast Specie	Effect	Inhibitory molecule	Inhibition	Affected microorganisms	Effect on <i>S. cerevisiae</i>	Reference
<i>Saccharomyces cerevisiae</i>	protein with a highly specific action spectrum and pH dependent	Killer toxin K1, K2		Sensitive yeasts	Sensitive strains	Studies on the nature of the killer factor produced by <i>Saccharomyces cerevisiae</i> . <a href="https://doi.org/10.1099/00221287-51-1-115">https://doi.org/10.1099/00221287-51-1-115</a>
<i>Pichia kluyveri</i> and <i>Candida pyralidae</i>  <i>Pichia kluyveri</i>	Antioxidative role (biofilm)  Antimicrobial  Production of Lactic acid		Against several yeasts and molds  Biofungicide with molds	Yeasts: <i>D. bruxellensis</i> <i>D. anomala</i> <i>Z. bailii</i> .  Fungi: <i>Botrytis cinerea</i> <i>Colletotrichum acutatum</i> <i>Rhizopus stolonifer</i>	-	The Use of <i>Candida pyralidae</i> and <i>Pichia kluyveri</i> to Control Spoilage Microorganisms of Raw Fruits Used for Beverage Production <a href="https://doi.org/10.3390/foods8100454">https://doi.org/10.3390/foods8100454</a>  Effects of <i>Pichia kluyveri</i> killer toxin on sensitive cells <a href="https://doi.org/10.1007/BF00444075">https://doi.org/10.1007/BF00444075</a>  High Potential of <i>Pichia kluyveri</i> and Other <i>Pichia</i> Species in Wine Technology <a href="https://doi.org/10.3390/ijms22031196">https://doi.org/10.3390/ijms22031196</a>
<i>Candida pyralidae</i>	Antimicrobial	Killer toxin	Against <i>Brettanomyces bruxellensis</i>	<i>Brettanomyces bruxellensis</i>		<i>Candida pyralidae</i> killer toxin disrupts the cell wall of <i>Brettanomyces bruxellensis</i> in red grape juice <a href="https://doi.org/10.1111/jam.13383">https://doi.org/10.1111/jam.13383</a>
<i>Candida intermedia</i>	Antimicrobial	Antimicrobial peptides	Against <i>Brettanomyces bruxellensis</i>	<i>Brettanomyces bruxellensis</i>		Novel antimicrobial peptides produced by <i>Candida intermedia</i> LAMAP1790 active against the wine-spoilage yeast <i>Brettanomyces bruxellensis</i> <a href="https://doi.org/10.1007/s10482-018-1159-9">https://doi.org/10.1007/s10482-018-1159-9</a>
<i>Metschnikowia pulcherrima</i>	Depletion of iron in the medium  SO <sub>2</sub> reduction	Pulcherriminic acid	Against several yeasts and molds  Biofungicide with molds	Yeasts: <i>Candida tropicalis</i> <i>Candida albicans</i> <i>Brettanomyces/Dekkera</i> <i>Hanseniaspora</i> <i>Pichia genera</i>  Fungi: <i>Botrytis cinerea</i> , <i>Penicillium</i>  <i>Alternaria</i> <i>Monilia spp.</i>	Non affected	Applications of <i>Metschnikowia pulcherrima</i> in Wine Biotechnology <a href="https://doi.org/10.3390/fermentation5030063">https://doi.org/10.3390/fermentation5030063</a>  Bio-Protection as an Alternative to Sulphites: Impact on Chemical and Microbial Characteristics of Red Wines <a href="https://doi.org/10.3389/fmicb.2020.01308">https://doi.org/10.3389/fmicb.2020.01308</a>  Non- <i>Saccharomyces</i> yeasts as bioprotection in the composition of red wine and in the reduction of sulfur dioxide <a href="https://doi.org/10.1016/j.lwt.2021.111781">https://doi.org/10.1016/j.lwt.2021.111781</a>  Antimicrobial activity of <i>Metschnikowia pulcherrima</i> on wine yeasts <a href="https://doi.org/10.1111/jam.12446">https://doi.org/10.1111/jam.12446</a>  Bioprotection on Chardonnay Grape: Limits and Impacts of Settling Parameters <a href="https://doi.org/10.1155/2022/1489094">https://doi.org/10.1155/2022/1489094</a>  Bio-protection in oenology by <i>Metschnikowia pulcherrima</i> : from field results to scientific inquiry <a href="https://doi.org/10.3389/fmicb.2023.1252973">https://doi.org/10.3389/fmicb.2023.1252973</a>
<i>Metschnikowia fructicola</i>	Control of ethyl acetate formation by apiculate yeasts during cold soak		apiculate yeasts	<i>H. uvarum</i>	Non affected	Influence of Select Non- <i>Saccharomyces</i> Yeast on <i>Hanseniaspora uvarum</i> Growth during Prefermentation Cold Maceration <a href="https://doi.org/10.5344/ajev.2020.20004">https://doi.org/10.5344/ajev.2020.20004</a>



<p><i>Metschnikowia pulcherrima</i> <i>Pichia kluyveri</i></p>	<p>Oxygen consumption</p>		<p>Protection against oxidation in pre-fermentation steps</p>		<p>Gerbaux et al., 2021 Revue des Enologues N° 179 partie 1 : Développement d'une levure de bioprotection des moûts blancs en phase préfermentaire : intérêt du concept.</p> <p>Biotechnological tools for reducing the use of sulfur dioxide in white grape must and preventing enzymatic browning: glutathione; inactivated dry yeasts rich in glutathione; and bioprotection with <i>Metschnikowia pulcherrima</i> <a href="https://doi.org/10.1007/s00217-023-04229-6">https://doi.org/10.1007/s00217-023-04229-6</a></p>
<p><i>Torulaspora delbrueckii</i></p>	<p>SO<sub>2</sub> reduction Control of enzymatic and chemical oxidation</p>		<p>Spoilage microorganisms</p>	<p>Non affected</p>	<p>Inoculation of <i>Torulaspora delbrueckii</i> as a bio-protection agent in winemaking <a href="https://doi.org/10.1016/j.foodres.2018.02.034">https://doi.org/10.1016/j.foodres.2018.02.034</a></p> <p>Non-<i>Saccharomyces</i> yeasts as bioprotection in the composition of red wine and in the reduction of sulfur dioxide <a href="https://doi.org/10.1016/j.lwt.2021.111781">https://doi.org/10.1016/j.lwt.2021.111781</a></p>
<p><i>Torulaspora delbrueckii</i> and <i>Metschnikowia pulcherrima</i></p>	<p>Antimicrobial SO<sub>2</sub> reduction Sensory effect O<sub>2</sub> consumption</p>		<p>Molds, apiculate yeasts, acetic acid bacteria</p>	<p>Decrease of fungal communities (HTS) and <i>Hanseniaspora uvarum</i> cultivable population</p>	<p>Innovative Use of Non-<i>Saccharomyces</i> in Bio-Protection: <i>T. delbrueckii</i> and <i>M. pulcherrima</i> Applied to a Machine Harvester <a href="https://doi.org/10.5344/catalyst.2020.20003">https://doi.org/10.5344/catalyst.2020.20003</a></p> <p>Population Dynamics and Yeast Diversity in Early Winemaking Stages without Sulfites Revealed by Three Complementary Approaches <a href="https://doi.org/10.3390/app11062494">https://doi.org/10.3390/app11062494</a></p> <p>Yeast and Filamentous Fungi Microbial Communities in Organic Red Grape Juice: Effect of Vintage, Maturity Stage, SO<sub>2</sub>, and Bioprotection <a href="https://doi.org/10.3389/fmicb.2021.748416">https://doi.org/10.3389/fmicb.2021.748416</a></p> <p>Sensory characterisation of wines without added sulfites via specific and adapted sensory profile <a href="https://doi.org/10.20870/oenone.2020.54.4.3566">https://doi.org/10.20870/oenone.2020.54.4.3566</a></p> <p>Bioprotection by non-<i>Saccharomyces</i> yeasts in oenology: Evaluation of O<sub>2</sub> consumption and impact on acetic acid bacteria <a href="https://doi.org/10.1016/j.ijfoodmicro.2023.110338">https://doi.org/10.1016/j.ijfoodmicro.2023.110338</a></p>
<p><i>Tetrapispora phaffii</i></p>	<p>SO<sub>2</sub> reduction</p>	<p>Killer toxin</p>	<p>Apiculate yeasts</p>	<p>Depending on starter strain</p>	<p>The zymocidal activity of <i>Tetrapispora phaffii</i> in the control of <i>Hanseniaspora uvarum</i> during the early stages of winemaking <a href="https://doi.org/10.1111/j.1472-765X.2009.02754.x">https://doi.org/10.1111/j.1472-765X.2009.02754.x</a></p> <p>Production of a lyophilized ready-to-use yeast killer toxin with possible applications in the wine and food industries <a href="https://doi.org/10.1016/j.ijfoodmicro.2020.108883">https://doi.org/10.1016/j.ijfoodmicro.2020.108883</a></p> <p>Evaluation of Recombinant Kpkt Cytotoxicity on HaCaT Cells: Further Steps towards the Biotechnological Exploitation Yeast Killer Toxins <a href="https://doi.org/10.3390/foods10030556">https://doi.org/10.3390/foods10030556</a></p>



<i>Lachancea thermotolerans</i>	Acidification/pH Increased effect of SO <sub>2</sub>	Lactic acid	Control of malolactic fermentation Effects on <i>Brettanomyces</i>	LAB <i>Aspergillus carbonarius</i> <i>Brettanomyces</i>	Non affected	<i>Lachancea thermotolerans</i> Applications in Wine Technology <a href="https://doi.org/10.3390/fermentation4030053">https://doi.org/10.3390/fermentation4030053</a>
<i>Wickerhamomyces anomalus</i> (formerly <i>Pichia/Hansenula anomala</i> )	Bind to β-1,3 and β-1,6 glucans	Killer Toxins of Broad Spectrum KTCF20 by the strain W. <i>anomalus</i> Cf20	Against several yeasts	Dekkera/ <i>Brettanomyces</i> <i>P. guilliermondii</i> <i>P. membranifaciens</i>	Non affected	Challenges of the Non-Conventional Yeast <i>Wickerhamomyces anomalus</i> in Winemaking <a href="https://doi.org/10.3390/fermentation4030068">https://doi.org/10.3390/fermentation4030068</a> Purification and Characterization of WA18, a New Mycocin Produced by <i>Wickerhamomyces anomalus</i> Active in Wine Against <i>Brettanomyces bruxellensis</i> Spoilage Yeasts <a href="https://doi.org/10.3390/microorganisms9010056">https://doi.org/10.3390/microorganisms9010056</a>
<i>Wickerhamomyces anomalus</i> and <i>Metschnikowia pulcherrima</i>	Antimicrobial		Against several spoilage yeasts			Selection of Native Non-Saccharomyces Yeasts with Biocontrol Activity against Spoilage Yeasts in Order to Produce Healthy Regional Wines <a href="https://doi.org/10.3390/fermentation5030060">https://doi.org/10.3390/fermentation5030060</a>
<i>Wickerhamomyces anomalus</i> and <i>K. wickerhamii</i>	Antimicrobial	Killer toxin	Against <i>Brettanomyces bruxellensis</i>	<i>Brettanomyces bruxellensis</i>	Depending on starter strain	Selection of Native Non-Saccharomyces Yeasts with Biocontrol Activity against Spoilage Yeasts in Order to Produce Healthy Regional Wines <a href="https://doi.org/10.1111/jam.13121">https://doi.org/10.1111/jam.13121</a>
<i>K. wickerhamii</i>	Antimicrobial	Killer toxin	Against <i>Brettanomyces bruxellensis</i>	<i>Brettanomyces bruxellensis</i>	Antimicrobial	<i>Kluyveromyces wickerhamii</i> killer toxin: purification and activity towards <i>Brettanomyces</i> / <i>Dekkera</i> yeasts in grape must <a href="https://doi.org/10.1111/j.1574-6968.2010.02194.x">https://doi.org/10.1111/j.1574-6968.2010.02194.x</a>
<i>Aureobasidium pullulans</i>	High-affinity iron-chelating molecules	Liamocins Siderophores chitinase glucanase	Exclusion of fungal adhesion sites, competition for nutrients and production of antagonistic metabolites or lytic enzymes	<i>Botrytis cinerea</i>	Non affected	The Multiple and Versatile Roles of <i>Aureobasidium pullulans</i> in the Vitivicultural Sector <a href="https://doi.org/10.3390/fermentation4040085">https://doi.org/10.3390/fermentation4040085</a>



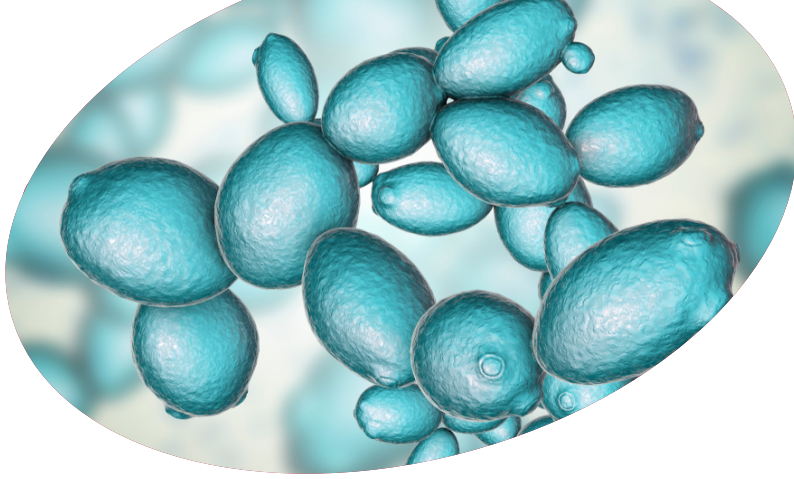


<p><i>Torulaspora delbrueckii</i>/ <i>Lachancea thermotolerans</i></p>	<p>Mixed inoculum in red winemaking exerted a comparable effect to that by SO<sub>2</sub></p>		<p>For LAB bioprotective effect comparable to that of SO<sub>2</sub>.</p> <p>For AAB only in the early stages of fermentation</p>	<p>Apiculate yeasts (<i>H. uvarum</i>)</p> <p>Lactic acid bacteria</p>		<p>Bioprotective Effect of a <i>Torulaspora delbrueckii</i>/<i>Lachancea thermotolerans</i>-Mixed Inoculum in Red Winemaking, <a href="https://doi.org/10.3390/fermentation8070337">https://doi.org/10.3390/fermentation8070337</a></p>
<p><b>Bacteria</b></p>						
<p><i>Oenococcus oeni</i></p>	<p>co-inoculation of selected yeast and selected wine bacteria to shorten the lag phase AF-MLF.</p> <p>Inoculation post AF with selected wine bacteria to shorten the duration o spontaneous MLF start</p> <p>Bacteriophages releases by some strains of <i>O.oeni</i></p>	<p>Lysin/holin</p>	<p><i>O. oeni</i> can suppress <i>Brettanomyces</i> growth</p> <p>Quick MLF helps to decrease the risk of spoilage LAB growth</p>	<p>Spoilage lactic acid bacteria, <i>Brettanomyces</i></p> <p>Other and spontaneous <i>Oenococcus oeni</i> strains sensitive to the bacteriophage released</p>		<p>Influence of Inoculation with Malolactic Bacteria on Volatile Phenols in Wines <a href="https://doi.org/10.5344/ajev.2009.60.2.233">https://doi.org/10.5344/ajev.2009.60.2.233</a></p> <p>Effet biocontrôle des bactéries lactiques sur la croissance de <i>Brettanomyces</i> et la production de phénols volatils dans le vin rouge <a href="https://search.oeno.tm.fr/search/article/9f952d23-7dc2-4a85-b55b-87b9281c299f">https://search.oeno.tm.fr/search/article/9f952d23-7dc2-4a85-b55b-87b9281c299f</a></p> <p>Gerbaux, V., Thomas, J., Briffox, C., Matéo, A. (2020). The advantage of using lactic acid bacteria for the biopreservation of wines against <i>Brettanomyces</i>. <i>Revue Française d'œnologie</i> ; 301 : 28-31</p> <p>Jaomanjaka, F. Diversité Des Bactériophages Infectant La Bactérie Lactique <i>Oenococcus Oeni</i>, Responsable de La Fermentation Malolactique Des Vins. These de doctorat, Bordeaux, 2014.</p> <p>Philippe, C. Bactériophages Infectant La Bactérie Lactique <i>Oenococcus Oeni</i> : Diversité et Rôles Dans l'écosystème œnologique. PhD Thesis, 2017.</p>
<p><i>Lactoplantibacillus plantarum</i></p>	<p>co-inoculation of selected yeast and selected wine bacteria to shorten the lag phase AF-MLF</p>	<p>Lactic acid Organic acids</p> <p>Acetic acid bacteria</p>		<p><i>Brettanomyces</i></p> <p><i>Acetobacter, aceti</i></p> <p><i>Acetobacter,</i> <i>Gluconobacter</i></p>	<p>Non affected</p>	<p><i>Lactobacillus plantarum</i>, a New Biological Tool to Control Malolactic Fermentation: A Review and an Outlook <a href="https://doi.org/10.3390/beverages6020023">https://doi.org/10.3390/beverages6020023</a></p> <p>Bioprotective Effect of <i>Pichia kluyveri</i> and <i>Lactiplantibacillus plantarum</i> in Winemaking Conditions <a href="https://doi.org/10.5344/ajev.2022.22008">https://doi.org/10.5344/ajev.2022.22008</a></p> <p>A Metagenomic-Based Approach for the Characterization of Bacterial Diversity Associated with Spontaneous Malolactic Fermentations in Wine <a href="https://doi.org/10.3390/ijms20163980">https://doi.org/10.3390/ijms20163980</a></p>



**Table 2.** Non exhaustive. But can be considered as some good practices of application with selected microorganisms.

Biocompatibility	Acidification	Persistence	Effect	References
<i>Lachancea thermotolerans</i> with <i>M pulcherrima</i> & <i>Saccharomyces cerevisia</i>	Higher than <i>L. thermotolerans</i> alone	During all fermentation	High biocompatibility	Biocompatibility in Ternary Fermentations With <i>Lachancea thermotolerans</i> , Other Non- <i>Saccharomyces</i> and <i>Saccharomyces cerevisiae</i> to Control pH and Improve the Sensory Profile of Wines From Warm Areas <a href="https://doi.org/10.3389/fmicb.2021.656262">https://doi.org/10.3389/fmicb.2021.656262</a>
<i>Lachancea thermotolerans</i> with <i>T delbrueckii</i> & <i>Saccharomyces cerevisiae</i>	Medium, lower than <i>L. thermotolerans</i> alone			
<i>Lachancea thermotolerans</i> with <i>H vineae</i> & <i>Saccharomyces cerevisiae</i>	Higher than <i>L. thermotolerans</i> alone	During all fermentation	Nutrient competition, thiamine depletion	
<i>Metschnikowia pulcherrima</i> and <i>Lachancea thermotolerans</i>				Synergetic Effect of <i>Metschnikowia pulcherrima</i> and <i>Lachancea thermotolerans</i> in Acidification and Aroma Compounds in Airén Wines <a href="https://doi.org/10.3390/foods11223734">https://doi.org/10.3390/foods11223734</a>



## **CONCLUSION**

In this document, different approaches and applications regarding bioprotection in winemaking are expressed. The definition of bioprotection, its scope, microorganisms and their initial population used, timing, temperature and dose of the application are outlined. A detailed table with some potential applications is provided with their corresponding references. As the tools of bioprotection are rapidly evolving, the latest advancements need to be followed and evaluated when their application is considered for wine production.

## **FURTHER REFERENCES ON BIOPROTECTION**

Pladeau, V., Pic, L., Cottreau, P., Richard, N. Bioprotection et gestion des fermentations alcooliques en bio. Résultats d'expérimentations en Languedoc-Roussillon (région Occitanie). November 2019.





Thanks, follow us.  
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