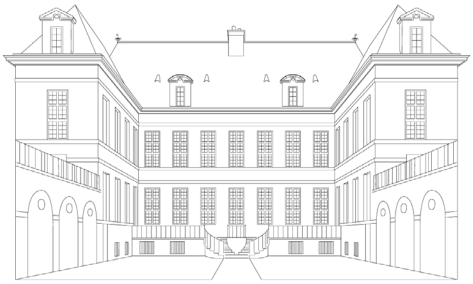
USE OF BIOPROTECTION STRAINS IN WINEMAKING

OIV



OIV 100

International Year of Vine and Wine 1924 • 2024

DISCLAIMER

OIV collective expertise documents are not submitted to the Step Procedure for Examining Resolutions and can under no circumstances be treated as OIV resolutions. Only resolutions adopted by the Member States of the OIV have an official character. This document has been drafted by the Oenology Commission's "Microbiology" (MICRO) Expert Group.
The current document has been drafted and developed on the initiative of the OIV and constitutes a collective expert document.

ISBN 978-2-85038-096-9
OIV - International Organisation of Vine and Wine
12, Parvis de l'UNESCO
F-21000 Dijon - France www.oiv.int
E-mail: oeno@oiv.int

COORDINATORS

Neslihan IVIT, OIV Oenology & Methods of Analysis Head of Unit
Jean-Claude RUF, OIV Scientific Coordinator

AUTHORS

Adriaan OELOFSE (ZA)
Angela CAPECE (IT)
Antonio MORATA (ES)
Christian VON WALLBRUNN (DE)
Guido BALDESCHI (IT)
Herve ALEXANDRE (FR)
Isabelle MASNEUF (FR)
Marina BELY (FR)
Maurizio CIANI (IT)
Nathalie SIECZKOWSKI (OENOPPIA)
Patricia TAILLANDIER (FR)
Raphaëlle TOURDOT-MARECHAL (FR)
Stéphane LA GUERCHE (OENOPPIA)
Sylvie COLLAS (FR)

ACKNOWLEDGEMENTS

Daniela Costa, OIV Communication Officer - layout

TABLE OF CONTENTS

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_2 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 ($<10e^4$ CFU/mL for yeasts, $<10e^2$ for bacteria). In terms of niche occupation, bioprotection is less effective at advanced maturity, although still possible.

SO_2 **resistance:** Some strains are only resistant to low levels of SO_2 (max. 30 mg/l). Bioprotection and SO_2 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.

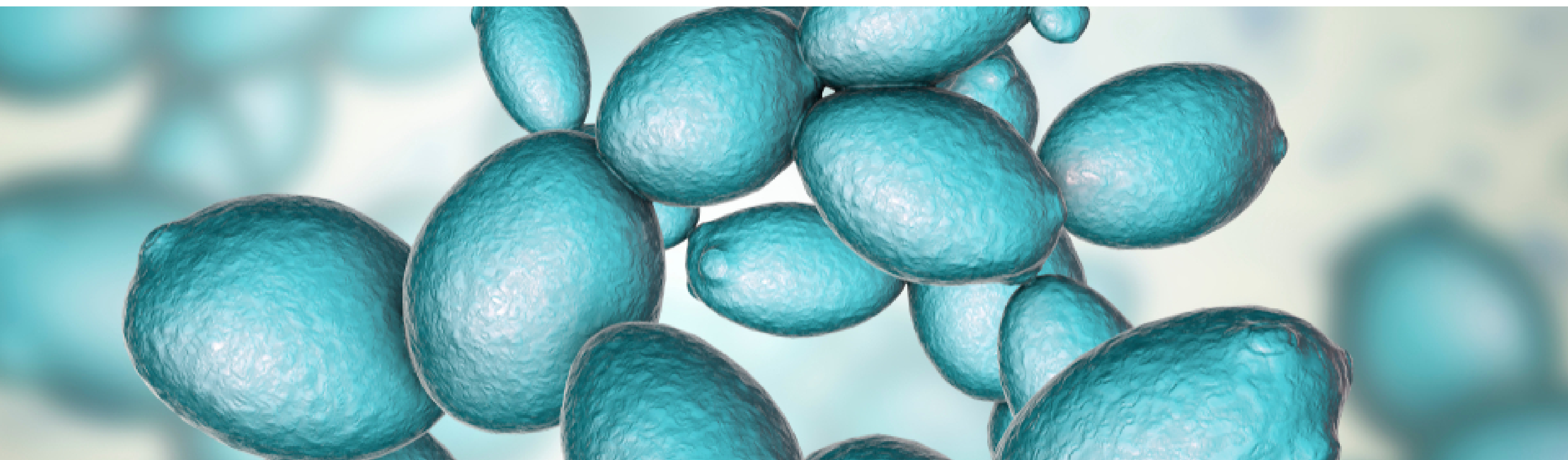
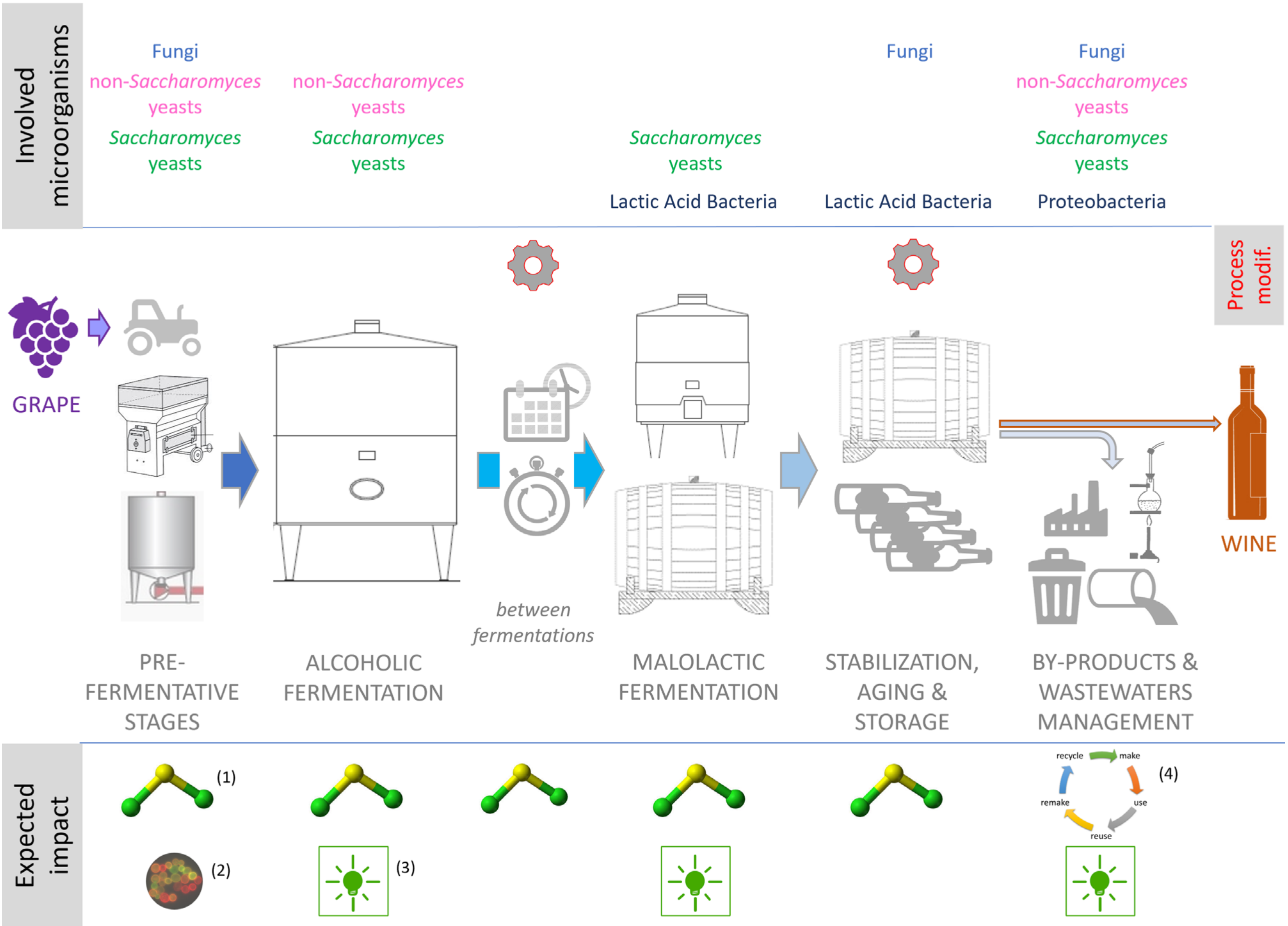


Fig A1. From: Nardi, T. Microbial Resources as a Tool for Enhancing Sustainability in Winemaking. Microorganisms 2020, 8, 507. https://doi.org/10.3390/microorganisms8040507



(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 S. cerevisiae	Reference
Saccharomyces cerevisiae	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 *Saccharomyces cerevisiae*. https://doi.org/10.1099/00221287-51-1-115
Pichia kluyveri and *Candida pyralidae* *Pichia kluyveri*	Antioxidative role (biofilm) Antimicrobial Production of Lactic acid		Against several yeasts and molds Biofungicide with molds	Yeasts: *D. bruxellensis* *D. anomala* *Z. bailii.* *Fungi:* *Botrytis cinerea* *Colletotrichum acutatum* *Rhizopus stolonifer*	-	The Use of *Candida pyralidae* and *Pichia kluyveri* to Control Spoilage Microorganisms of Raw Fruits Used for Beverage Production https://doi.org/10.3390/foods8100454 Effects of *Pichia kluyveri* killer toxin on sensitive cells https://doi.org/10.1007/BF00444075 High Potential of *Pichia kluyveri* and Other *Pichia* Species in Wine Technology https://doi.org/10.3390/ijms22031196
Candida pyralidae	Antimicrobial	Killer toxin	*Against Brettanomyces bruxellensis*	*Brettanomyces bruxellensis*		*Candida pyralidae* killer toxin disrupts the cell wall of *Brettanomyces bruxellensis* in red grape juice https://doi.org/10.1111/jam.13383
Candida intermedia	Antimicrobial	Antimicrobial peptides	*Against Brettanomyces bruxellensis*	*Brettanomyces bruxellensis*		Novel antimicrobial peptides produced by *Candida intermedia* LAMAP1790 active against the wine-spoilage yeast *Brettanomyces bruxellensis* https://doi.org/10.1007/s10482-018-1159-9
Metschnikowia pulcherrima	Depletion of iron in the medium SO_2 reduction	*Pulcherriminic* acid	Against several yeasts and molds Biofungicide with molds	Yeasts: *Candida tropicalis* *Candida albicans* *Brettanomyces/Dekkera* *Hanseniaspora* *Pichia genera* *Fungi:* *Botrytis cinerea, Penicillium* *Alternaria* *Monilia spp.*	Non affected	Applications of *Metschnikowia pulcherrima* in Wine Biotechnology https://doi.org/10.3390/fermentation5030063 Bio-Protection as an Alternative to Sulphites: Impact on Chemical and Microbial Characteristics of Red Wines https://doi.org/10.3389/fmicb.2020.01308 Non-*Saccharomyces* yeasts as bioprotection in the composition of red wine and in the reduction of sulfur dioxide https://doi.org/10.1016/j.lwt.2021.111781 Antimicrobial activity of *Metschnikowia pulcherrima* on wine yeasts https://doi.org/10.1111/jam.12446 Bioprotection on Chardonnay Grape: Limits and Impacts of Settling Parameters https://doi.org/10.1155/2022/1489094 Bio-protection in oenology by *Metschnikowia pulcherrima*: from field results to scientific inquiry https://doi.org/10.3389/fmicb.2023.1252973
Metschnikowia fructicola	Control of ethyl acetate formation by apiculate yeasts during cold soak		apiculate yeasts	*H. uvarum*	Non affected	Influence of Select Non-*Saccharomyces* Yeast on *Hanseniaspora uvarum* Growth during Prefermentation Cold Maceration https://doi.org/10.5344/ajev.2020.20004

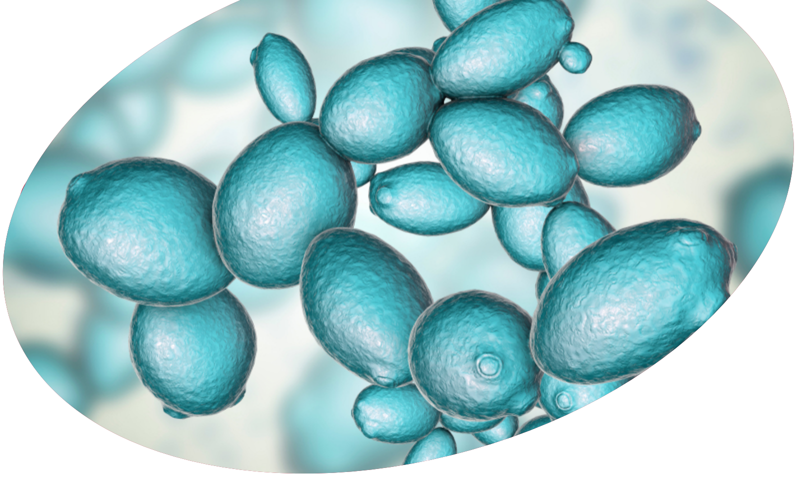
Metschnikowia pulcherrima *Pichia kluyveri*	Oxygen consumption		Protection against oxidation in pre-fermentation steps			Gerbaux et al., 2021 Revue des Œnologues N° 179 partie 1 : Développement d'une levure de bioprotection des moûts blancs en phase préfermentaire : intérêt du concept. Biotechnological 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 *Metschnikowia pulcherrima* https://doi.org/10.1007/s00217-023-04229-6
Torulaspora delbrueckii	SO_2 reduction Control of enzymatic and chemical oxidation		Spoilage microorganisms		Non affected	Inoculation of *Torulaspora delbrueckii* as a bio-protection agent in winemaking https://doi.org/10.1016/j.foodres.2018.02.034 Non-*Saccharomyces* yeasts as bioprotection in the composition of red wine and in the reduction of sulfur dioxide https://doi.org/10.1016/j.lwt.2021.111781
Torulaspora delbrueckii and *Metschnikowia pulcherrima*	Antimicrobial SO_2 reduction Sensory effect O_2 consumption		Molds, apiculate yeasts, acetic acid bacteria	Decrease of fungal communities (HTS) and *Hanseniaspora uvarum* cultivable population		Innovative Use of Non-*Saccharomyces* in Bio-Protection: *T. delbrueckii* and *M. pulcherrima* Applied to a Machine Harvester https://doi.org/10.5344/catalyst.2020.20003 Population Dynamics and Yeast Diversity in Early Winemaking Stages without Sulfites Revealed by Three Complementary Approaches https://doi.org/10.3390/app11062494 Yeast and Filamentous Fungi Microbial Communities in Organic Red Grape Juice: Effect of Vintage, Maturity Stage, SO_2, and Bioprotection https://doi.org/10.3389/fmicb.2021.748416 Sensory characterisation of wines without added sulfites via specific and adapted sensory profile https://doi.org/10.20870/oeno-one.2020.54.4.3566 Bioprotection by non-*Saccharomyces* yeasts in oenology: Evaluation of O_2 consumption and impact on acetic acid bacteria https://doi.org/10.1016/j.ijfoodmicro.2023.110338
Tetraposispora phaffii	SO_2 reduction	Killer toxin	Apiculate yeasts		Depending on starter strain	The zymocidial activity of *Tetrapisispora phaffii* in the control of *Hanseniaspora uvarum* during the early stages of winemaking https://doi.org/10.1111/j.1472-765X.2009.02754.x Production of a lyophilized ready-to-use yeast killer toxin with possible applications in the wine and food industries https://doi.org/10.1016/j.ijfoodmicro.2020.108883 Evaluation of Recombinant Kpkt Cytotoxicity on HaCaT Cells: Further Steps towards the Biotechnological Exploitation Yeast Killer Toxins https://doi.org/10.3390/foods10030556

Lachancea thermotolerans	Acidification/pH Increased effect of SO_2	Lactic acid	Control of malolactic fermentation Effects on *Brettanomyces*	LAB *Aspergillus carbonarius* *Brettanomyces*	Non affected	*Lachancea thermotolerans* Applications in Wine Technology https://doi.org/10.3390/fermentation4030053
Wickerhamomyces anomalus (formerly Pichia/ Hansenula anomala)	Bind to ß-1,3 and ß-1,6 glucans	Killer Toxins of Broad Spectrum KTCf20 by the strain W. anomalus Cf20	Against several yeasts	*Dekkera/Brettanomyces* *P. guilliermondii* *P. membranifaciens*	Non affected	Challenges of the Non-Conventional Yeast *Wickerhamomyces anomalus* in Winemaking https://doi.org/10.3390/fermentation4030068 Purification and Characterization of WA18, a New Mycocin Produced by *Wickerhamomyces anomalus* Active in Wine Against *Brettanomyces bruxellensis* Spoilage Yeasts https://doi.org/10.3390/microorganisms9010056
Wickerhamomyces anomalus and Metschnikowia pulcherrima	Antimicrobial		Against several spoilage yeasts			Selection of Native Non-*Saccharomyces* Yeasts with Biocontrol Activity against Spoilage Yeasts in Order to Produce Healthy Regional Wines https://doi.org/10.3390/fermentation5030060
Wickerhamomyces anomalus and K. wickerhamii	Antimicrobial	Killer toxin	Against *Brettanomyces bruxellensis*	*Brettanomyces bruxellensis*	Depending on starter strain	Selection of Native Non-*Saccharomyces* Yeasts with Biocontrol Activity against Spoilage Yeasts in Order to Produce Healthy Regional Wines https://doi.org/10.1111/jam.13121
K. wickerhamii	Antimicrobial	Killer toxin	Against Brettanomyces bruxellensis	*Brettanomyces bruxellensis*	Antimicrobial	*Kluyveromyces wickerhamii* killer toxin: purification and activity towards *Brettanomyces/ Dekkera* yeasts in grape must https://doi.org/10.1111/j.1574-6968.2010.02194.x
Aureobasidium pullulans	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	*Botrytis cinerea*	Non affected	The Multiple and Versatile Roles of *Aureobasidium pullulans* in the Vitivinicultural Sector https://doi.org/10.3390/fermentation4040085

Torulaspora delbrueckii/ Lachancea thermotolerans	Mixed inoculum in red winemaking exerted a comparable effect to that by SO_2		For LAB bioprotective effect comparable to that of SO_2. For AAB only in the early stages of fermentation	Apiculate yeasts (*H. uvarum*) Lactic acid bacteria		Bioprotective Effect of a *Torulaspora delbrueckii/Lachancea thermotolerans*-Mixed *Inoculum* in Red Winemaking, https://doi.org/10.3390/fermentation8070337
Bacteria						
Oenococcus oeni	co-inoculation of selected yeast and selected wine bacteria to shorten the lag phase AF-MLF. Inoculation post AF with selected wine bacteria to shorten the duration o spontaneous MLF start Bacteriophages releases by some strains of O.*oeni*	Lysin/holin	O. *oeni* can suppress *Brettanomyces* growth Quick MLF helps to decrease the risk of spoilage LAB growth	Spoilage lactic acid bacteria, *Brettanomyces* Other and spontaneous *Oenococcus oeni* strains sensitive to the bacteriophage released		Influence of Inoculation with Malolactic Bacteria on Volatile Phenols in Wines https://doi.org/10.5344/ajev.2009.60.2.233 Effet biocontrôle des bactéries lactiques sur la croissance de *Brettanomyces* et la production de phénols volatils dans le vin rouge https://search.oeno.tm.fr/search/article/9f952d23-7dc2-4a85-b55b-87b9281c299f Gerbaux, V., Thomas, J., Briffox, C., Matéo, A. (2020). The advantage of using lactic acid bacteria for the biopreservation of wines against *Brettanomyces*. Revue Francaise d'œnologie ; 301 : 28-31 Jaomanjaka, F. Diversité Des Bactériophages Infectant La Bactérie Lactique *Oenococcus Oeni*, Responsable de La Fermentation Malolactique Des Vins. These de doctorat, Bordeaux, 2014. Philippe, C. Bactériophages Infectant La Bactérie Lactique *Oenococcus Oeni* : Diversité et Rôles Dans l'écosystème œnologique. PhD Thesis, 2017.
Lactoplantibacillus plantarum	co-inoculation of selected yeast and selected wine bacteria to shorten the lag phase AF-MLF	Lactic acid Organic acids Acetic acid bacteria		*Brettanomyces* *Acetobacter, aceti* *Acetobacter, Gluconobacter*	Non affected	*Lactobacillus plantarum*, a New Biological Tool to Control Malolactic Fermentation: A Review and an Outlook https://doi.org/10.3390/beverages6020023 Bioprotective Effect of *Pichia kluyveri* and *Lactiplantibacillus plantarum* in Winemaking Conditions https://doi.org/10.5344/ajev.2022.22008 A Metagenomic-Based Approach for the Characterization of Bacterial Diversity Associated with Spontaneous Malolactic Fermentations in Wine https://doi.org/10.3390/ijms20163980

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

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

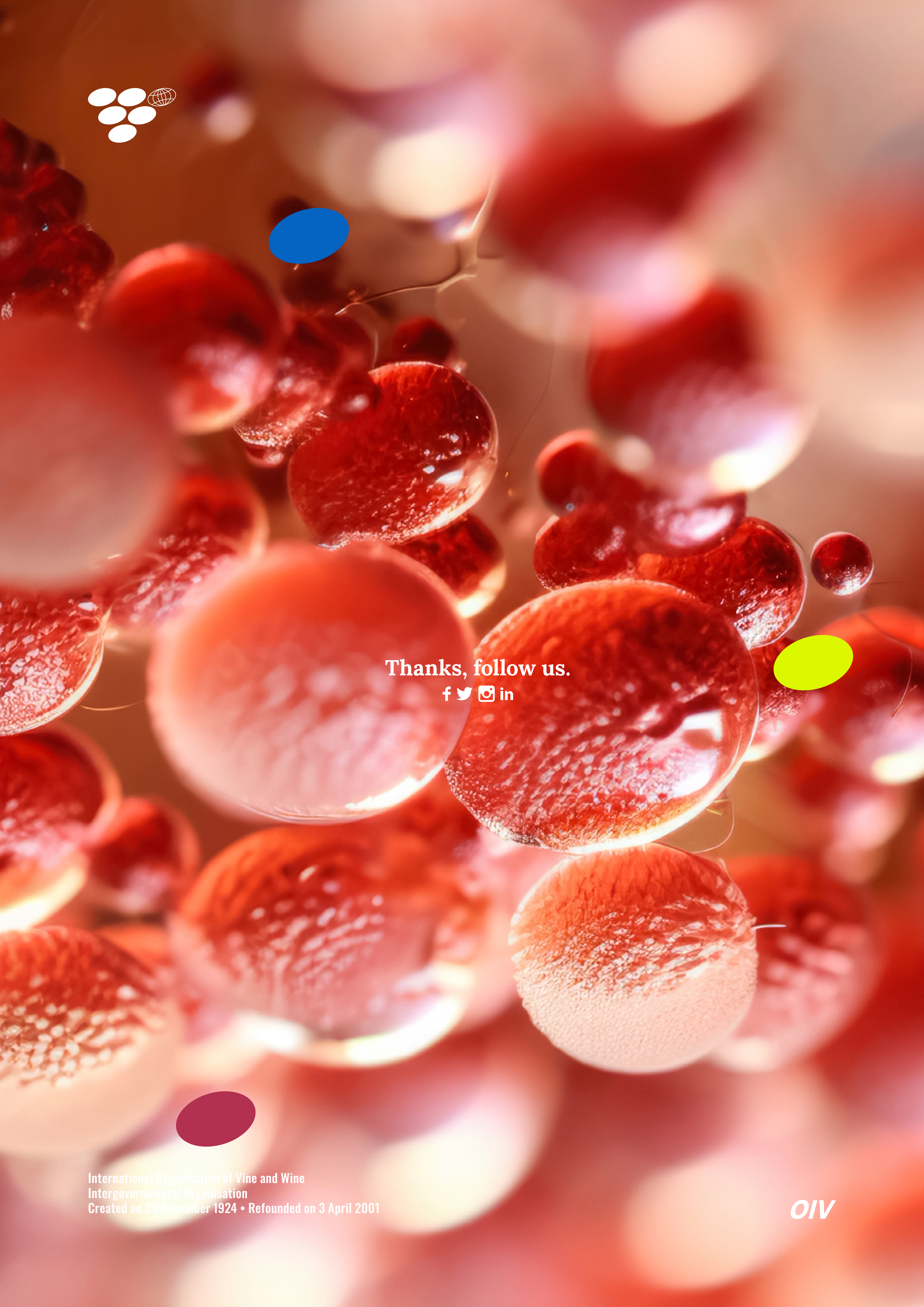


CONCLUSION

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., Cottereau, P., Richard, N. Bioprotection et gestion des fermentations alcooliques en bio. Résultats d’expérimentations en Languedoc-Roussillon (région Occitanie). November 2019.



Thanks, follow us.

International Organisation of Vine and Wine
Intergovernmental Organisation
Created on 29 November 1924 • Refounded on 3 April 2001

OIV