Bioprotection

Research and Development

What is Bioprotection?

Bioprotection refers to the use of natural products to control pests and diseases. This approach has gained significant interest globally, particularly in the wine industry, due to increasing consumer demand for preservative-free and allergen-free wines.

Bioprotection in winemaking aims to enhance wine quality by minimising or eliminating chemical additives, and naturally inhibiting unwanted microorganisms found on grapes or in the must.

History and the need for Alternatives to SO_2

Historically, sulphur dioxide (SO_2) was the only solution available to winemakers for wine protection due to its antioxidant and antimicrobial properties. However, it can trigger adverse health reactions in sensitive consumers.
With growing health consciousness, scientists are exploring alternatives to SO_2, and winemakers are seeking to reduce sulphites in wines.

Application of *Non-Saccharomyces* Yeast Products

Non-Saccharomyces yeast products, added to juice in the pre-fermentation stage or to grapes during harvesting, can colonise all contact surfaces and prevent unwanted microorganisms. Recent research has shown that some *non-Saccharomyces* yeast species can provide antimicrobial protection on white and red grape varieties as an alternative to sulphites, and can limit the development of natural microbiota, in particular potential spoilage microorganisms like *Brettanomyces* and acetic acid bacteria in a manner similar to the addition of sulphites.

Antimicrobial Activity of *Metschnokowia pulcherrima*

A study evaluated the antimicrobial activity of seven strains of *Metschnokowia pulcherrima* against 114 yeast strains. Results showed that *M. pulcherrima* had no influence on the growth of *Saccharomyces cerevisiae* but effectively inhibited undesired wild spoilage yeasts.

Optimal Conditions for Bioprotective Effect

Application trials suggest that colder grape juice temperature improves bioprotectant implantation, indicating that these yeasts should be added during cold maceration for optimal bioprotective effect. *M. pulcherrima* strain was tested on Chardonnay must at different temperatures and settling times, showing a significant impact of temperature on the bioprotectant implantation.

Additional Properties of *Metschnikowia pulcherrima*

M. pulcherrima not only acts as a biocontrol agent but also produces secondary metabolites that can enhance the sensorial profile of the wine. It also has hydrolytic enzymatic activities.

Some *M. pulcherrima* strains have antioxidant properties, such as a high oxygen consumption rate and the ability to remove copper.

An AWRI study using **Pinnacle AWRI Bioprotect** showed a decrease in acetic acid concentrations when spiked with *Hanseniaspora uvarum*. (Figure 1).

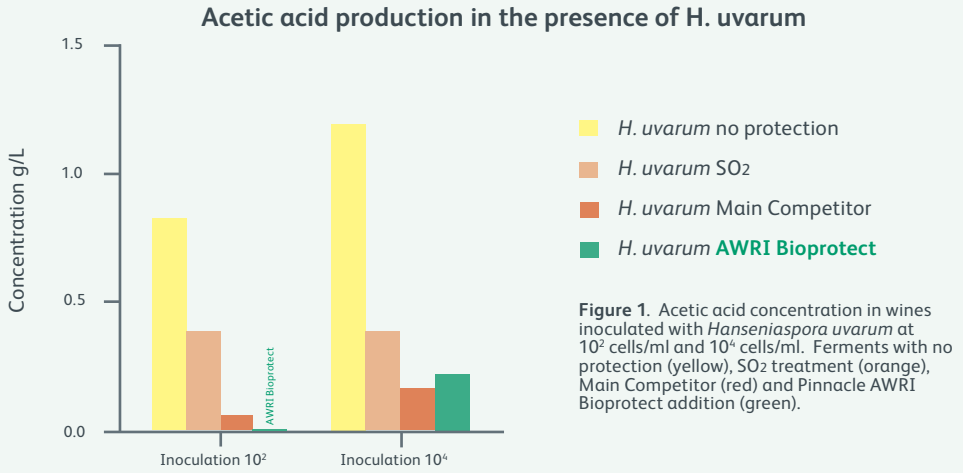

Figure 1. Acetic acid concentration in wines inoculated with *Hanseniaspora uvarum* at 10^2 cells/ml and 10^4 cells/ml. Ferments with no protection (yellow), SO_2 treatment (orange), Main Competitor (red) and Pinnacle AWRI Bioprotect addition (green).

Introduction to Pinnacle AWRI Bioprotect

Pinnacle AWRI Bioprotect is a *Metschnikowia pulcherrima* strain, isolated by The Australian Wine Research Institute (AWRI) for its bioprotection abilities. It displays low yeast assimilable nitrogen (YAN) uptake and requires a sequential inoculation with a strong *S. cerevisiae* yeast to complete fermentation due to its inherent alcohol tolerance of between 2% and 6% v/v ethanol.

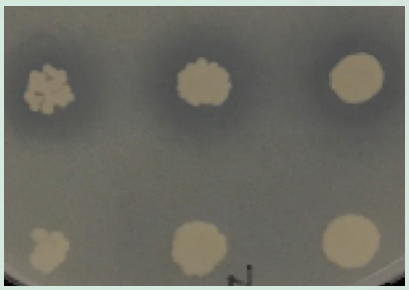

Figure 2: Bioprotective effect of *Metschnikowia pulcherrima* Pinnacle AWRI Bioprotect compared to a control *Saccharomyces cerevisiae* yeast on the growth of the spoilage organism *Brettanomyces bruxellensis*. Serial dilutions of the strains (10^{-1}, 10^{-2} and 10^{-3} from right to left) were spotted on nutrient plates. Clear zones of inhibition by Pinnacle AWRI Bioprotect can be seen.

Bioprotective Effect of Pinnacle AWRI Bioprotect

Pinnacle AWRI Bioprotect offers a natural method of wine protection. It inhibits unwanted microbial populations, effectively stopping their ability to influence the final aroma and flavour of the wine. The Institute de Ciencias de la Vid y del Vino (ICVV) conducted a study to produce a stable SO_2-free wine using Pinnacle AWRI Bioprotect in a sequential fermentation. The results showed that in contrast to SO_2 Pinnacle AWRI Bioprotect had no effect on the fermentation kinetics or malolactic fermentation (MLF) but seemed to enhance the wine's chemical compounds after 9 months of storage.

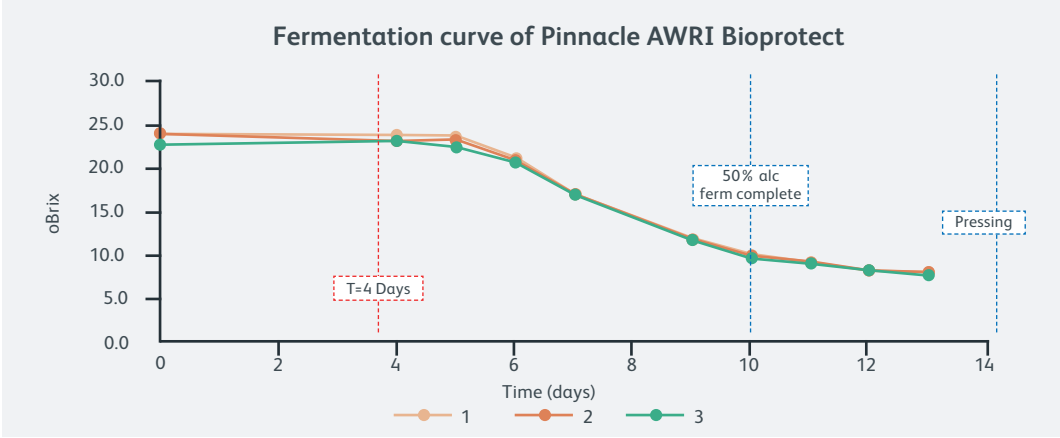

Figure 3.
Fermentation kinetic (Mean± standard deviation n = 2), Where 1 = SO_2 + *Saccharomyces*, 2 = no SO_2 + *Saccharomyces* and 3 = **Pinnacle AWRI Bioprotect** + *Saccharomyces*.

Pinnacle AWRI Bioprotect had no effect on malolactic fermentation (MLF); it seems that MLF occurred faster with Pinnacle AWRI Bioprotect compared to the fermentation with SO_2 which was the slowest. (Figure 4).

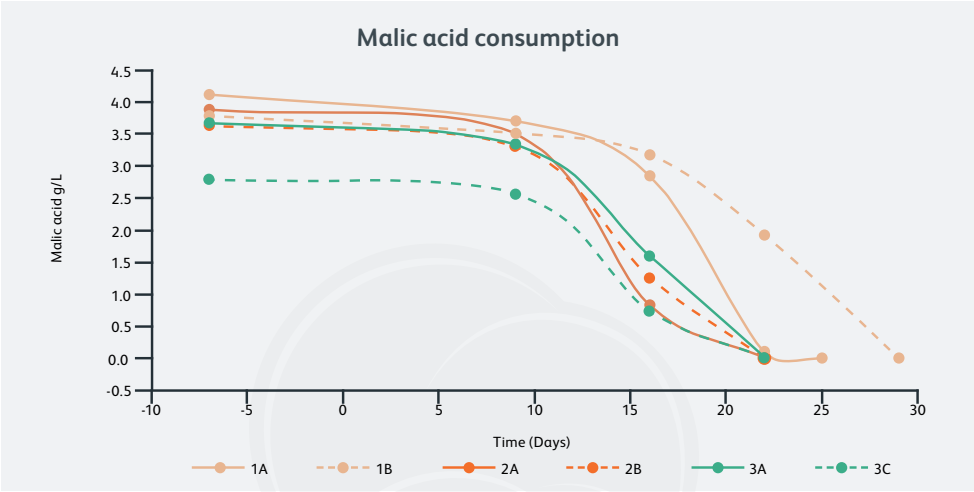

Figure 4.
Evolution of malic acid during the second half of the alcoholic fermentation and during the malolactic fermentation (Where 1A and 1B = a SO_2 + *Saccharomyces* + bacteria, where 2A and 2B = no SO_2 + *Saccharomyces* + bacteria and where 3A and 3C = **Pinnacle AWRI Bioprotect** + *Saccharomyces* + bacteria).

If the chemical compounds produced by each ferment are compared it seems that the wine produced with Pinnacle AWRI Bioprotect was as good if not better as the one produced with SO_2 after 9 months of storage.

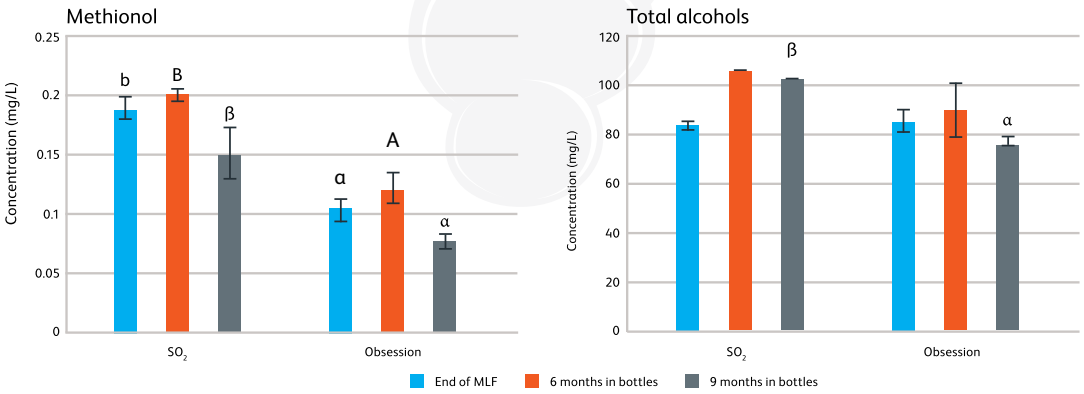

Figure 5.
Higher alcohols concentration (mg/L) in the wines at the end of malolactic fermentation (MLF) and after 6 and 9 months in bottles. All parameters are given as average values ± the standard deviations (n = 2). Different small, capital or Greek letters indicate significant differences between samples ($p \leq 0.05$) at the end of MLF, after 6 or 9 months in bottles, respectively. If there are no letters, it means that there were no significant differences between samples ($p > 0.05$).

Table. Enological parameters of the wines at the end of malolactic fermentation (MLF) and after 6 and 9 months in bottles. All parameters are given as average values ± the standard deviations (n = 2).

	Endo f MLF		6 months in bottles		9 months in bottles	
	SO_2	*Obsession*	*SO_2*	*Obsession*	*SO_2*	*Obsession*
Alcohol degree (% v/v)	13.75 ± 0.07 a	13.23 ± 0.25 a	-	-	-	-
pH	4.04 ± 0.06 a	3.95 ± 0.01 a	-	-	-	-
Total acidity (g/L)*	5.10 ± 0.11 a	5.23 ± 0.13 a	-	-	-	-
Malic acid (g/L)	n.d.	n.d.	-	-	-	-
Lactic acid (g/L)	2.62 ± 0.10 a	2.81 ± 0.01 a	-	-	-	-
Volatile acidity (g/L)**	0.50 ± 0.02 a	0.46 ± 0.06 a	0.46 ± 0.0 a	0.46 ± 0.06 a	0.49 ± 0.04 a	0.47 ± 0.10 a
YAN (mg N/L)	9 ± 2 a	8 ± 3 a	-	-	-	-
DO 420 nm	0.24 ± 0.01 a	0.23 ± 0.03 a	0.28 ± 0.02 a	0.27 ± 0.03 a	0.30 ± 0.02 a	0.29 ± 0.03 a
DO 520 nm	0.30 ± 0.02 a	0.30 ± 0.05 a	0.36 ± 0.04 a	0.35 ± 0.06 a	0.37 ± 0.05 a	0.37 ± 0.06 a
DO 620 nm	0.07 ± 0.00 a	0.06 ± 0.01 a	0.11 ± 0.01 a	0.10 ± 0.02 a	0.12 ± 0.01 a	0.12 ± 0.01 a
Color intensity (CI)	6.11 ± 0.27 a	5.85 ± 0.88 a	7.51 ± 0.74 a	7.21 ± 1.10 a	8.00 ± 0.74 a	7.73 ± 1.01 a
Total polyphenol index (TPI)	43.70 ± 0.69 a	41.66 ± 4.02 a	43.40 ± 0.42 a	41.50 ± 3.54 a	42.04 ± 0.49 a	40.15 ± 3.36 a
Total anthocyanins (mg/L)	553.3 ± 15.2 a	499.4 ± 67.1 a	226.1 ± 11.1 a	204.4 ± 35.9 a	73.1 ± 0.3 a	66.1 ± 8.5 a
Total polyphenols (mg/L)	1718.6 ± 63.7 a	1626.5 ± 48.9 a	1626 ± 15.3 a	1626.5 ± 146.1 a	1536.8 ± 11.2 a	1458.2 ± 122.8 a

AWRI (10 g/hL). T4 days: Sc_1 = Maurivin AWRI796. TEAF: MLB = Pinnacle Malosafe. n.d.: not analysed. YAN: Yeast assimilable nitrogen. *As g/L of tartaric acid. **As g/L of acetic acid. For each moment (end of MLF, 6 and 9 months in bottles), different letters indicate significant differences between samples ($p \leq 0.05$).

Conclusion and Application in the Winery

The AWRI study mentioned above suggests that **Pinnacle AWRI Bioprotect** heavily influences the growth of different spoilage microorganisms during the pre-fermentation stage of the winemaking process. It can inhibit the growth of *H. uvarum* and limit the growth of *Brettanomyces bruxellensis*.

Pinnacle AWRI Bioprotect can be added to the harvest and winery equipment to ensure continuous colonisation of contact surfaces for the duration of harvest, preventing the accumulation of unwanted microorganisms. It can also be added directly to the must or juice during any of the pre-fermentative stages for better microbial control.

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