**PRE-FERMENTATIVE BIOPROTECTION**

# Gaïa™

Have confidence in Nature
to protect your juice and grapes

IOC
Révélons votre différence

# BIOPROTECTION: CONTROLLING THE LIVING THROUGH THE LIVING

From harvest to the tank, microorganisms responsible for the production of elevated acetic acid (VA) and other undesirable aroma compounds can rapidly multiple, especially under conducive conditions. This risk increases with the extended duration of pre-fermentation operations, such as grape transportation, cold pre-fermentation maceration, cold storage of juices or grape dehydration, and too high temperatures (> 8°C) or desire to reduce the use of $SO_2$. Climate variations and changes in viticultural practices also lead to maturity levels that accentuate the development of undesirable (spoilage) microorganisms.

The French Institute of Vine and Wine of Beaune has selected GAÏA™, a *Metschnikowia fructicola* yeast strain with no real fermentative power to fight against this harmful microflora. It occupies the ecological niche by limiting the proliferation of undesirable yeasts and minimises the risk of alcoholic fermentation starting too early. GAÏA™ can be used as a major tool to reduce the use of $SO_2$, either at the time of tank filling, or used at earlier stages (machine or grape hopper or press). It also facilitates the implantation of inoculated *S. cerevisiae* strains which lead the fermentation.

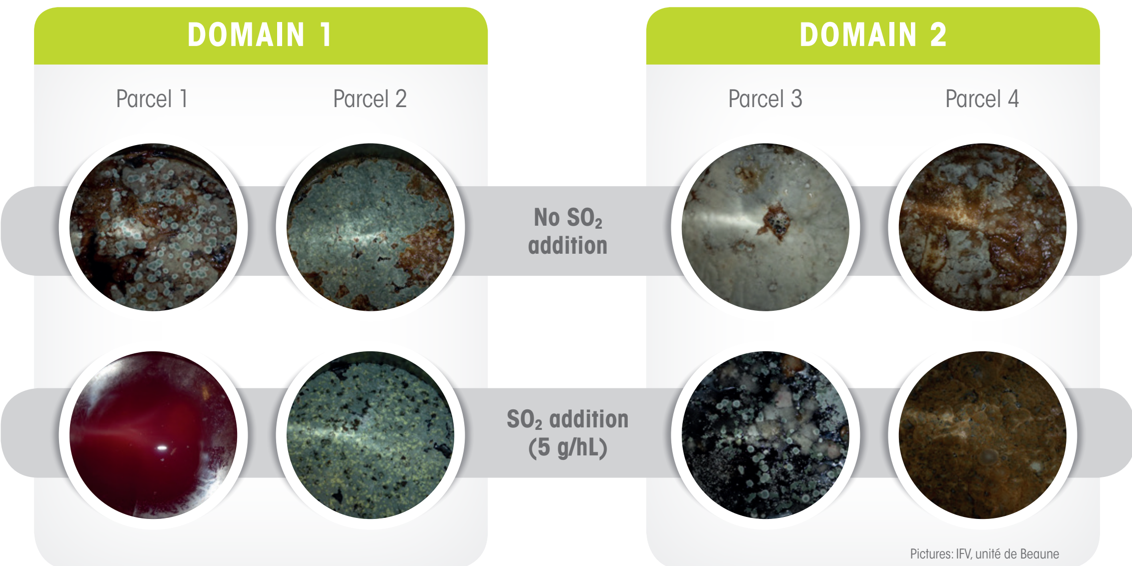
Faced with new social and technical challenges, this concept of bioprotection, biocontrol or «positive contamination» is destined to become a major lever for winemakers in their mastery of preferred grape processing.

# THE MAIN PRE-FERMENTATION RISKS

## THE MOLDS

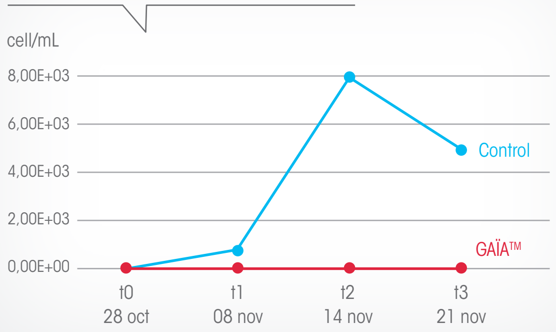
The flora at grape harvest is not as innocuous as one might think. Grapes pressed and incubated under sterile conditions (i.e. the flora only originating from the grape), the first development observed will be that of mold, without even starting fermentation after several days (Figure 1).

**Figure 1: Development of mold on the surface of grape must in the absence of contamination from the cellar or wine equipment (only flora of the grapes)** – Observation after 9 days at 20°C.

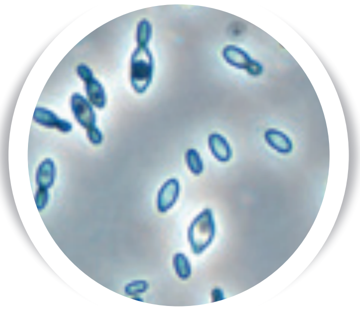


Beyond moulds observed in musts in tanks, which do not present any particular danger in real œnological conditions, there is an actual problem when air-drying grape bunches, e.g. for producing Amarone-type wine. During the weeks when drying is carried out in the winery prior to vinification of the grapes, significant growth of *Botrytis cinerea* often occurs, which can adversely affect the quality of the fruit. The *Mestchnikowia fructicola* GAÏA™ yeast makes it possible to keep this undesirable growth under control to a minimum level.

**Growth of *Botrytis cinerea* during raising**




## APICULATE YEASTS

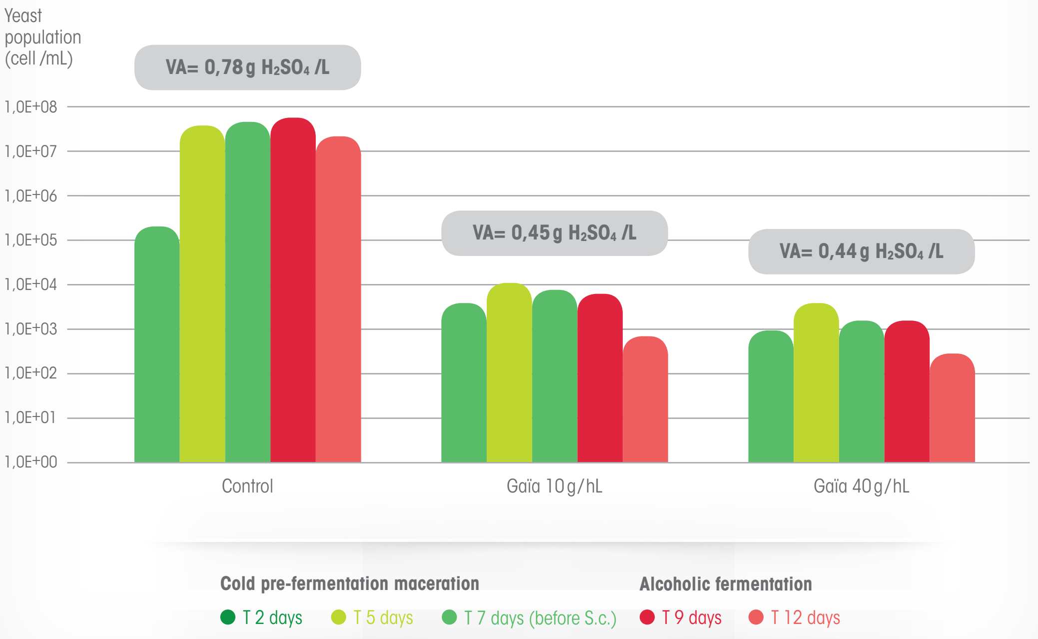


*Hanseniaspora uvarum* (also known as *Kloeckera apiculata*) is one of the most harmful representatives of grape yeast. It has a lemon-shaped morphology and many of its strains are responsible for very large increases in the level of volatile acidity (up to 4 times higher than *S. cerevisiae*) and ethyl acetate concentration (solvent-like aroma - up to 10 times more than *S. cerevisiae*).

This yeast is often the major species on healthy ripe grapes. It has low fermentation capacity (up to an average of 5-6% vol) but multiplies extremely fast, either during the transport of the grapes, or cold maceration. Low temperatures (below 15°C) favor their resistance to alcohol and we sometimes observe cases of dominance of this species at the end of alcoholic fermentation!

On certain grape harvests, this pre-fermentation development may be restricted by the presence of *Metschnikowia* yeasts, which are almost non-fermenting and do not produce acetic acid. GAÏA™ yeast, a *Metschnikowia fructicola*, conducts this bioprotection phenomenon (Figure 2).

**Figure 2: Evolution of *Hanseniaspora uvarum* populations and production of final acetic acid in the absence or presence of pre-fermentation bioprotection with GAÏA™**
Pinot noir (pasteurized must) pH 3.6, non $SO_2$ added - cold soak at 13°C



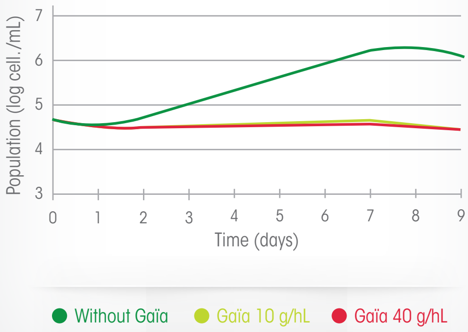
## *BRETTANOMYCES BRUXELLENSIS*

This yeast, responsible for phenol and medicinal aromas, contaminates wine mainly during aging or when waiting for completion of malolactic fermentation. Sometimes it develops very early in must during fermentation. GAÏA™ yeast could partially inhibit this early growth, however the best way to combat *Brettanomyces bruxellensis* is the co-inoculation for MLF with *O. oeni.*

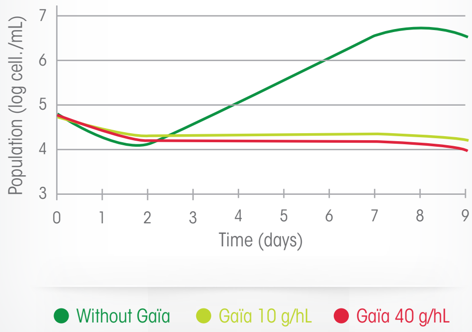
## ACETIC ACID BACTERIA

In general, the development of acetic acid bacteria is less common. However, high populations of insects such as vinegar fruit flies which can act as bacteria vectors, at harvest can lead to the production of high concentrations of volatile acidity. We have demonstrated the biocontrol effect exerted by GAÏA™ on the *Acetobacter* and *Gluconobacter* populations by mimicking this type of contamination under experimental conditions (Figure 4).

**Monitoring of the *Acetobacter* population at 16°C**
Pinot noir-Chardonnay (pasteurised must) pH 3.6, no $SO_2$ addition, pre-fermentation stage at 13°C for 7 days

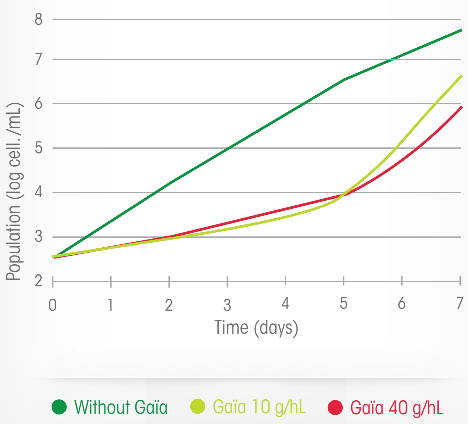



**Monitoring of the *Gluconobacter* population at 16°C**
Pinot noir-Chardonnay (pasteurised must) pH 3.6 no $SO_2$ addition, pre-fermentation stage at 13°C for 7 days




## UNWANTED FERMENTATION INITIATION

**Figure 5. Monitoring of the *Saccharomyces* population during the pre-fermentation phase at 13°C**
Pinot noir-Chardonnay (pasteurised must) pH 3.6 no $SO_2$ addition, pre-fermentation stage at 13°C for 7 days




Under certain circumstances where the pre-fermentation phase is extended, the risk of growth of native *Saccharomyces* is inevitable, up to a level where they can trigger alcoholic fermentation earlier than desired. These risks are all the more important when the temperature is high and the level of $SO_2$ is low; for example during skin maceration, cold pre-fermentation maceration, or cold storage of grape juice.

Sensory and technological impact of an early unwanted fermentation initiation include: difficulties in juice settling, need to filter regularly, refrigeration costs, production of $SO_2$ by the native *Saccharomyces* yeast, lack of control of the wine sensory profile, difficulties of implantation of the selected yeast *S. cerevisiae*, and the probability of stuck or sluggish fermentation.

GAÏA™ helps to slow the development of these indigenous *Saccharomyces* populations (Figure 5), slowing down the start of fermentation. The temperature of the must is a key factor in the effectiveness of this technique, because the colder the juice, the more *Metschnikowia fructicola* is favored compared to *Saccharomyces*.

# GAÏA™ CAN BE USED IN A RANGE OF APPLICATIONS

### At machine harvesting

**Anticipate and protect your grapes at the earliest**
To avoid any proliferation of microorganisms from the harvest and during transport to the cellar.

### During transport of picked fruit

**Manage long transport times**
Suitable for temperatures >15°C, long transport times, extended waiting times and degraded sanitary conditions.

### On the grapes during drying

**Limit development of *Botrytis cinerea* during drying process (e.g. amarone)**
Reduces the development of rot often observed in the drying chambers.

### At the reception of grapes in the cellar

**Protect the must for the duration of the pre-fermentation phases**
Allows to fight against spoilage microorganisms or the early start of fermentation.

### When filling cold pre-fermentation maceration tanks

**Fight against rising volatile acidity with limited fermentation start**
Fight against *Hanseniaspora uvarum* with limited fermentation start, allowing for extraction of anthocyanins in the aqueous phase.

### In the wine press

**Limit the risks of starting fermentation and reduce $SO_2$ additions**
Limits the development of fermentative yeasts, especially in case of lower $SO_2$ adds, to allow a good clarification after pressing.

### Out of the press of the white juice for sparkling wines (traditional method)

**Limit spoilage and control the sensory profile**
With global warming (e.g. leading to higher than desired pH), and the desire to limit $SO_2$ concentrations, an addition of GAÏA™ at the beginning of filling the settling tank helps to reduce yeast or acetic acid bacteria growth, and limits unwanted aromatic development that will harm the elegance and finesse of sparkling wines made in the traditional method.


### In the wine press for white juices or rosé musts

**Limit the risk of early start of fermentation and reduce $SO_2$ additions.**
Limits the development of fermentative yeasts, especially in case of lower $SO_2$ additions, to allow a good clarification after pressing.

### Before yeasting for sparkling base wines (Closed tank method)

**Limit fermentation start and ethanol production during must warming**
The juice warming phase (stored at low temperature) for fermentation can last up to 72 h, resulting in undesirable microbiological development, especially sources of large amounts of acetaldehyde. The addition of GAÏA™ in the cold juice before heating avoids the triggering of unwanted fermentation.

### On juice during storage

**Protection of juice during storage or transport over extended periods**
Maintain the juice in an optimal condition for its use during the year, and to reduce expenses (e.g. refrigeration, filtration) to avoid unwanted fermentation.

# BIOPROTECTION WITH GAÏA™: USE IN WHITE GRAPE JUICE

The effectiveness of the bioprotection by GAÏA™ depends on various factors: temperature, time of addition, initial microbial load on the grapes, duration of the pre-fermentation phases, homogeneity of the GAÏA™ in the juice/grapes, dose used, and content of sulphites.

Unlike $SO_2$ or heat treatments, GAÏA™ does not act a priori as a fungicide or bactericide, but prevents the initial yeast and bacterial populations from developing to a level conducive for alterations (e.g. elevated VA) or unwanted fermentation.

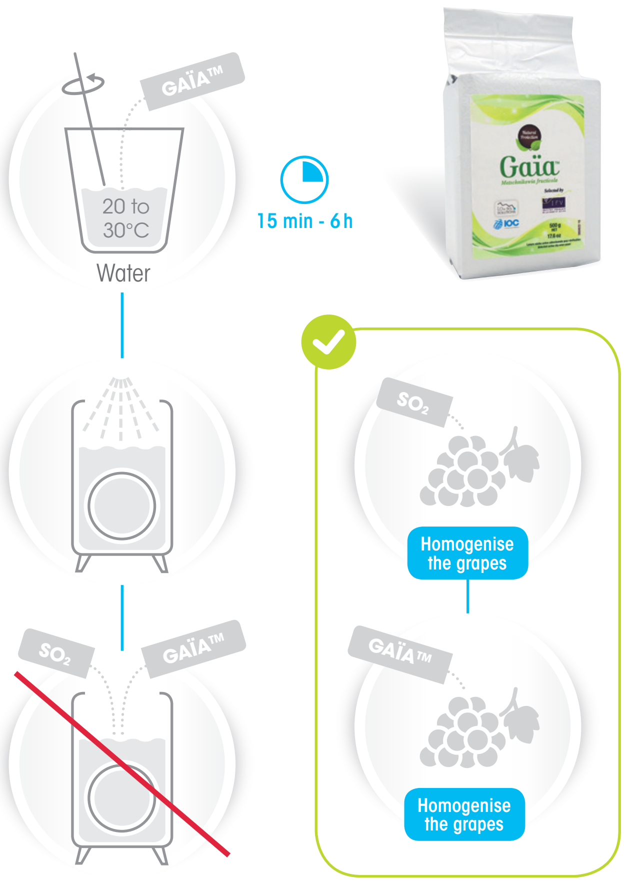
Thus, the earlier the inoculation of GAÏA™, the more effective this biocontrol will be.

The temperature of the grapes/juice is a key factor for the effectiveness of GAÏA™; the colder the better the biocontrol. The effectiveness of the biocontrol by GAÏA™ *Metschnikowia fructicola* is compared to *Saccharomyces cerevisiae*, and the better will be the biocontrol it will have with respect to an undesired departure in fermentation.

| GRAPE MUST/JUICE TEMPERATURE | 0°C | 8°C | 12°C | 16°C |
|---|---|---|---|---|
| **Average indicative duration of non-fermentative phase** | Several weeks or months without any fermentative activity | 7-10 days or more, then very limited fermentative activity | 4-5 days then very limited fermentative activity | 2 days then very limited fermentative activity |

## INSTRUCTIONS FOR USE

- GAÏA™ yeast is rehydrated in 10 times its weight in non-chlorinated and unsweetened water at 20 to 30°C. Mix well to break and disperse any lumps. Allow to stand for 15 minutes. Direct rehydration in the must is not recommended.
- This suspension retains excellent viability for 6 hours and can therefore be prepared in advance at the winery when to be used in the vineyard. If this suspension to be used later, add must after 45 minutes of rehydration to prolong its life.
- Slightly mix the suspension to homogenise it before spreading it evenly over the grapes / must (sprayer, watering can, incorporation as filling progresses, reassembly just after inoculation...). Ensure good mixing (homogenisation) of the GAÏA™ in the grapes/must to ensure good colonisation over the whole volume.
- The bioprotection by GAÏA™ makes it possible to replace the $SO_2$, or to complement its action. Avoid adding GAÏA™ simultaneously with an $SO_2$ addition. Homogenization of $SO_2$ (max. 5 g/hL) in the grapes / must mass prior to the addition of GAÏA™ is necessary. In the same way, if GAÏA™ is added just before $SO_2$ addition, ensure perfect homogenization of this yeast in the mass before incorporating and homogenizing the $SO_2$.
- Because of its extremely low nitrogen consumption, the use of GAÏA™ does not require any change in the nutritional protocol of the yeast used thereafter to conduct the fermentation.
- After the desired period of time for the cold soak/pre-fermentation, warm and inoculate the grape must with *Saccharomyces cerevisiae*.



## SPECIFIC RECOMMENDATIONS FOR EACH APPLICATION

| | DOSE | COMMENTS |
|---|---|---|
| **Cold soak of red grapes** | 7-10 g/hL if good sanitary state Up to 25 g/hL if high pH, high microbial load or presence of acetic acid bacteria. | Homogeneous addition throughout the filling of the tank (or before). Temperatures >15°C not recommended to avoid an initiation of AF. Temperatures <12°C desirable. |
| **Skin contact of white grapes** | 7-10 g/hL. | Addition during the filling of the press, homogeneously, or before. |
| **Maceration with / of grape solids (white and rosé)** | 7 g/hL. | Addition during filling of the tank (or before). Temperature <10°C. |
| **Harvest bioprotection before transportation to the winery** | 7-10 g/hL. | Automatic machine spray system on harvest machine or hand addition as the grape-gondolas are filled. An extra addition in the bottom of the grape-gondola is sometimes desirable in case of release of juice under the pressure of the weight of the grapes. |
| **Storage and transportation of cold juice** | 10 g/hL (20 g/hL if high microbial pressure in winery or temperature > 0°C) | Add during the filling. Do not warm the juice before inoculation and always keep the juice at a temperature of 0°C. |
| **Warming of the must before triggering a delayed fermentation (Charmat method)** | 10 g/hL. | Addition in cold must (0°C) just before warming, or addition even before, during the storage of the juice. |

## TO LEARN MORE ABOUT GAÏA™ AND BIOPROTECTION


Gerbaux V., 2016: Mastery of the course of alcoholic fermentation under practical conditions. French Review of oenology 277: 9-11.

Gerbaux V., Davanture I., Guilloteau A., Julien-Ortiz A., Raginel F. and Silvano A., 2015: Cold pre-fermentation maceration of red wines - *Metschnikowia pulcherrima* GaïaMP98.3: a new microbiological pathway to secure the process and optimize the sensory impact. Oenologists Review 155: 29-33.

Kurtzman C. and Droby S., 2001: M*etschnikowia fructicola*, a new ascosporic yeast with potential for biocontrol of postharvest fruit rots. System. Appl. Microbiol. 24, 395-399.

# FAQ

## How does GAÏA™ biocontrol work?

It would appear that there are several mechanisms involved. The high level of population implanted when inoculated with GAÏA™ and its ability to survive in a poor environment, such as the surface of the grape or in a low-temperature must, give it a competitive edge in comparison to other flora. There is also competition for thiamine: GAÏA™ deprives *Hanseniaspora uvarum* – which needs it in large quantities – of thiamine. GAÏA™ is also known to be able to produce pulcherriminic acid, which complexes with iron, rendering it unavailable for fungi such as *Botrytis cinerea*.

## How can GAÏA™ yeast multiply without consuming practically any nitrogen, or sugars? How does it survive?

Inoculating with GAÏA™ does not necessarily produce any yeast growth: the implanted population is sufficient to carry out biocontrol. It is often more a question of survival than multiplication. GAÏA™ consumes a negligible quantity of nitrogen (approximately 10 mg/L).

## Does GAÏA™ have any sensory impact?

By reducing the use of $SO_2$, GAÏA™ helps eliminate sulphite-related sensory risks, such as reductive odours, masking fruitiness or dryness in mouth. It also helps limit high content of volatile acidity and ethyl acetate that sometimes occur in the pre-fermentation process. GAÏA™ however hasn't been selected to have a direct sensory impact.

## Can GAÏA™ protect me from *Brettanomyces* contaminations during ageing?

GAÏA™ is effective in the pre-fermentation process. When there is alcohol, and unlike *Brettanomyces*, the GAÏA™ population drops sharply. Although GAÏA™ may help bring about a slight decrease in the population of *Brettanomyces* during the pre-fermentation phase, biocontrol ceases when there is alcoholic fermentation and growth of *Brettanomyces* may then start up again under favourable conditions. The best way of avoiding such growth is to use œnological bacteria, especially when they are inoculated early in fermentation (co-inoculation).

## Can I use GAÏA™ without rehydration?

It's possible, but we don't recommend it. Rehydrating yeast helps revitalize them, and in environments where there is little water, such as uncrushed grapes, it is all the more important. Moreover, by having a suspension of GAÏA™, it assists to maximise dispersion of the yeast population to quickly colonise the whole of the grape environment or must to be protected.

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