Keep it great! with bioprotection

Chr.Hansen, Anne-Claire Bauquis
Bioprotection is a natural way to inhibit microbial contaminants (yeast, mold and bacteria). This biocontrol not only helps you to prevent flavor defects, it also helps you to build and protect your brand!
Keep your brand strong - with bioprotection

- Bioprotection is the art of using natural microbial food cultures to inhibit unwanted contaminants – preventing food spoilage or enhancing food safety.

- Fermentation by food cultures has been known from ancient times as a natural way of preserving food.

- We use our superior knowledge of food microbiology to identify and select the best of the good bacteria/yeast to be used for bioprotection.

- Bioprotection enhances the effectiveness of a good sanitation program but is never an alternative to good cleaning and hygienic design of the production.

Highlight: While it takes time and diligence to create a strong brand, everything can be compromise in one moment with consumers communicating on bad brand experiences via social media.
Bioprotection can keep it great for fresh dairy, cheese, meat and wine.
How many consumers look at the label?

66% of consumers worldwide check food and drink labels before purchasing a product

61% of these check for artificial preservatives

Source: Euromonitor
Keep it great! For Dairy
Keep It great with FreshQ®

FreshQ® cultures are natural food cultures selected especially to protect dairy products.

FreshQ® helps to prevent spoilage, extends shelf life and protects the goodness of your fresh dairy and cheese products – even after it has been opened. It makes it easier for you to deliver a consistent and enjoyable brand experience.
Yeast & mold spoilage

Most common source of microbial spoilage in fermented dairy products. A whole world of risks can create a peak of contamination:

- High production capacity utilization / Season – pressure on sanitation program
- Use of ingredient with higher risk of contamination (ex. Fruit preps)
- Weather conditions
- Use of higher risk processes
- Deviation from standard production
  ....and many more

Highlight: Yeast and mold contamination levels and types are not constant
Very strong antifungal effect of FreshQ®

Extents of outgrowth in 18% sour cream after 45 days @ 7°C/45°F

Reference

FreshQ®

Reference

FreshQ®

P. crustosum  P. glabrum  P. brevicompactum  P. commune  P. solitum  A. versicolor

Extents of outgrowth in 18% sour cream after 45 days @ 12°C/54°F
FreshQ® inhibits yeast

Example: *Debaryomyces hansenii* (added at 50 cfu/g) in 18% sour cream stored at 7°C/45°F
What can FreshQ® do for you?

FreshQ® cultures can benefit you in four distinct ways:

- **Go natural**
- **Take control**
- **Stay fresh**
- **Extend shelf life**
Keep it great! For Meat
Keep It great with SafePro®

SafePro® cultures are natural food cultures selected especially to protect meat & prepared food products.

SafePro® helps prevent spoilage, extend shelf life and maintain or improve the safety and the goodness of your meat, salmon & salads – even after it has been opened. It makes it easier for you to deliver a consistent and enjoyable brand experience.
Pathogens

Most common safety concern in meat and prepared food. Numerous sources and risks to manage:

- Large variability in influx of pathogens through the raw materials – soil, intestinal, ..
- High production capacity utilization – pressure on sanitation program
- Creation of biofilm – increased sanitation challenge
- Many points of cross contamination

….and many more

In 2013, 1763 cases of human listeriosis were reported in EU. All cases (99%) were hospitalized – 191 persons died (EFSA, 2015)
A **tried and tested** method

- Challenge tests designed to prove the effect of SafePro® against a cocktail of *Listeria monocytogenes* strains in real meat and salmon products.

- Proves whether there is an effect under constant contamination and the results are easy to read.

- Results are based on relevant contamination levels and realistic storage conditions and duration – all according to international and/or local standards.

- In addition, it is suggested to run an industrial trial with SafePro® to confirm the absence of negative or even improved sensory impact.

**Fresh fact:** Field trials are necessary to prove that SafePro® can help you achieve the benefits under the realistic conditions in your production and market.
SafePro® inhibits *Listeria monocytogenes*

Example: *Listeria monocytogenes* in bacon stored at 20°C for 29 days
Safepro® takes control of the eco-system

Example: Flora composition before and after treatment and at end of shelf life

Before B-LC-77 inoculation
- Chryseobacterium sp.
- Lactobacillus sp.
- Microbacterium lacticum
- Pediococcus acidilactici
- Ralstonia pickettii
- Staphylococcus equorum
- Staphylococcus carnosus
- Rhodovulum sp.
- Others (<1% of the sequences)

After B-LC-77 inoculation
- Chryseobacterium sp.
- Lactobacillus sp.
- Microbacterium lacticum
- Pediococcus acidilactici
- Ralstonia pickettii
- Staphylococcus equorum
- Staphylococcus carnosus
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- Others (<1% of the sequences)

End of shelf life
- Chryseobacterium sp.
- Lactobacillus sp.
- Microbacterium lacticum
- Pediococcus acidilactici
- Ralstonia pickettii
- Staphylococcus equorum
- Staphylococcus carnosus
- Rhodovulum sp.
- Others (<1% of the sequences)
Keep it great with SafePro®

What can SafePro® do for you? SafePro® cultures can benefit you in four distinct ways:

- Go natural
- Take control
- Keep it safe
- Extend shelf life
For wine, bio-control has similar focus to dairy:

- Keep a fresh and clean taste
- Avoid spoilage contaminants
- Enable extended storage/maturation without flavor deviation
Keep It great with Viniflora®

Viniflora® cultures are natural food cultures selected especially to control fermentation processes preserving the varietal character of the grapes.

Viniflora® helps to prevent the growth of undesirable flora and protects the flavor potential of the wine during its maturation. It makes it easier for you to deliver a consistent and enjoyable brand experience.
Bacteria, yeast & mold spoilage

In wine, spoilage can originate from many kinds of micro-organisms. A whole world of risks can create a peak of contamination:

- The process itself: Use of high moisture fruit without pasteurization leads to a danger of undesirable micro-organisms
- Weather conditions and time from harvest to start of fermentation
- Use of high risk processes like uncontrolled fermentation with indigenous flora
- Seasonal activity leads to pressure on workers and possible lapses
  ....and many more
What can Viniflora® do for winemakers?

Bioprotection can benefit you in three distinct ways:

**Go natural**

**Take control**

**Improve bottom line**
Keep it great!

Go natural

Keep the label clean with no added SO$_2$ - or else minimize its use.

If you improve quality and consistency the natural way, you can keep your label clean.

Use Viniflora® to make sure your wines are appreciated by as many people as possible, reducing the use of SO$_2$ in your wine.
What do consumers think about sulfites?

Sulfites are unwanted by most of consumers

- Chemical preservative
- Toxic and allergenic
- Gives headache
- Damages flavors in wine
Sulfite consumption from wine may exceed acceptable daily intake

Toxical chemical agent, submitted to regulation: US (2003) and Europe (1994/2014) with labeling obligation (when >10 ppm), and low Acceptable daily intake (ADI) value of 56 mg/day (for a 80 Kg man).

- Red dry wines: = 150 mg/l i.e 18 mg per glass*
- Dry white and Rosé = 250 mg/l i.e 30 mg per glass
- Sparking wines = 210 mg/l i.e. 25 mg per glass
- Sweet wines = 300 mg/l i.e. 36 mg per glass

*glass=0.12 l
Sulfites inhibit the technological microorganisms

In wine, sulfites have been used for years for many reasons:

- **Antioxidant**: Avoid oxidation by combining the oxygen.

- **Preservatives (antibacterial and antifungal agent)**: **Control** unwanted microflora but also **inhibit** technological yeast and bacteria

- The preservative functionality can be replace by a suitable use of bioprotective culture

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**Sensitivity of selected microorganisms to active SO₂** (BEECH-1979 and USSEGLIO-TOMASSET-1982)

<table>
<thead>
<tr>
<th>Sensitivity threshold</th>
<th>Active SO₂ (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fermentative yeast</td>
<td>0.4-3</td>
</tr>
<tr>
<td>Lactic acid bacteria</td>
<td>2.3-3.7</td>
</tr>
</tbody>
</table>
Keep it great!

Take control

Use Viniflora® bioprotective cultures to make sure there is no deviation in the process and to keep your wine character, from vintage to vintage.
What risks in case of non-controlled process?

RISK ZONE

**Take control**

**What risks in case of non-controlled process?**

- **Variable raw material**
  - High/low sugar
  - High/low malic acid
  - Too high or too low pH
  - Possible high contaminants
  - Possible oxidation
  - Possible high sulfites
  - Possible pesticides

- **Variable microbial activity**
  - Difficult or inadequate fermentation
  - Growth of unwanted microorganisms

- **Non mastered metabolites production**
  - Biogenic Amines
  - Off flavor /unclean flavor
  - Volatile acidity

- **Possible issues on stock**
  - Undesired flora: LAB, Brett
  - Undesired flavor/Unclean
  - Breaking bottles
  - High sulfites
  - High alcohol
  - Down grading value
  - Brand damage

- **Harvest**
- **Crush**
- **Alc**
- **Mlf**
- **Maturation/storage**
- **Consumption**
There is hardly no technological microflora in grapes

Microflora in a sample of Merlot grapes studied by means of metagenomic analysis

• **Fungi**
  - Saccharomyces
  - Penicillium
  - Kabatiella
    - (endophytic fungus living in flower, leaves and stems)
  - Aspergillus
    - (plant pathogen and endophyte)
  - Cladosporium

• **Bacteria**
  - Loriellopsis
    - Family of cyanobacteria or blue-green algae
  - Crinalium
    - Family of cyanobacteria or blue-green algae
  - Iphinoe
    - Family of cyanobacteria or blue-green algae

• Wine technological species are quasi absent from grape juice and must (or in very limited concentration)
• The grape juice is dominated by plant and environmental related molds and bacteria
Selected bioprotective cultures help to control the whole process

- Reduce initial concentration of contaminants
- Prevent contaminant from growing
- Increase acidity and reduce alcohol
- Avoid use of SO₂

For wine improvement:
- Avoid growth of undesirable flora (Bacteria: Lactobacilli, Pediococci, non selected Oenococcus or yeast: Brettanomyces)
- Avoid off-flavor and biogenic amines linked to contaminants
- Avoid use of SO₂
“Pre” and “post” alcohol fermentation - opportunities for process improvement and safety

**Crush**

“Pre-fermentation”
For must improvement

- Early colonization of must/wine
- Anti-oxidation
- Lactic acid production
- Booster of *S. cerevisiae*/*Oenococcus oeni*
- Inhibition of undesired bacteria and yeast

+ Production of aroma precursors
  Mouth feeling improvement

**Alc**

Malolactic cultures: for improvement and stabilisation of wine

- Colonization of the medium
- Inhibition of *Brettanomyces* and Lactic acid bacteria

**Mlf**

Maturation/storage

**Consumption**

Protect your future
Stock value & Brand value:

- Flawless wines
- Reliable process/Vintage to vintage consistency
- Positive health impact
- Natural solution

Take control
How does bioprotection take place? - 4 mechanisms may occur

**Inhibition through competition for nutrients**

- Better adaptation to the medium conditions.

**Formation of antagonistic substances** by the protective strain that limit the development of other microorganisms. Each molecule has a specific spectrum of activity.

**Cell signal (communication)** by the protective strain that limit the development of other microorganisms.

**Inhibition through competition for space.** Space in the suspension can be limited, and the fastest duplicating strain could get a survival advantage.

Keep it great!

**Examples**

- **Yeast:** Concerto™ and FrootZen™
- **Bacteria:** CH16 and CiNe™
How to bioprotect your wine with Concerto™?

- Strong colonization
- Production of lactic acid → higher TA & lower pH → bioprotection
- Reduces the growth of wild bacteria and fungi → specific inhibition towards Acetic bacteria
- Good synergy with *S. cerevisiae* → fast implementation of suitable microflora

**Lachancea thermotolerans** (former *Kluyveromyces thermotolerans*)
- Improves acid balance
- Possible reduction of alcohol
Viniflora® Concerto™ takes control of the Fungi eco-system

Example of flora composition before and after inoculation, until the final wine step: domination of Lachancea and Saccharomyces

Each color represents a specie
Increased acidity in wines with Concerto™

Example: Increase of Non-volatile acidity and decrease of pH with Viniflora® Concerto™

A higher acidity ensures a protection against unwanted flora.
How to bioprotect your wine with FrootZen™?

- Anti-oxidation
- Production of lactic acid → higher TA & lower pH → bioprotection
- Inhibit the growth of bacteria at an early stage
- Booster of *S. cerevisiae* → fast implementation of suitable microflora
- Additional interest: Flavor molecules and precursors

*Pichia Kluyzeri*

- Amazing “tutti fruity”
FrootZen™ reduces dissolved oxygen concentration in the grape juice during fermentation

FrootZen™ avoid grape oxidation and enable to reduce the must

P. Kluyveri: Frootzen™
VL3: S. cerevisiae

Ferments are anaerobic within 2-4 hours of inoculation, no dissolved oxygen can be detected afterwards.
FrootZen can be used to manage bacteria growth during fermentation

The similarity of the sequences day 0-2 indicate that there is no real bacteria growth – Indicating that FrootZen can be used to manage bacteria growth in must and during the fermentation
How to bioprotect your wine with CH16?

- Strong wine colonization
- No production of biogenic amines
- Fast consumption of malic acid
  ➔ Protection towards *Brettanomyces*
  ➔ Protection towards unwanted lactic acid bacteria (*Lactobacilli, Pediococci, Leuconostocs, Oenococcus*)

Viniflora® CH16
*Oenococcus oeni*
Viniflora® CH16 takes control of the bacteria eco-system

Example of flora composition before and after inoculation, until the final wine step: domination of *Oenococcus*. A high growth helps to prevent contaminants development.

Each color represents a genus.
No production of biogenic amines with **Viniflora®** cultures

Example of histamine production in Spontaneous fermentation conditions versus a mastered fermentation with *Oenococcus oeni* from Viniflora® range.

In some countries, there is a maximum histamine allowed in final wine (Germany for instance: <2.0 ppm histamine ).
How to bioprotect your wine with CiNe™?
- the stabilization power without buttery flavor

• Strong colonization

• No production of biogenic amines

• Fast consumption of malic acid
  → Protection towards *Brettanomyces*
  → Protection towards unwanted lactic acid bacteria (*Lactobacilli, Pediococci, Leuconostocs, Oenococcus*)

• No diacetyl flavor if unwanted → case of certain white and rosé wines
CiNe™ - a specific metabolism for a crisp fruit flavor

Product performance

- **Malic acid**
  - Decrease in concentration over time
  - CiNe and Oenos exhibit different profiles

- **Citric acid**
  - Low concentration levels
  - No citrate uptake leads to no citrate degradation

- **Diacetyl**
  - Below sensory threshold
  - CH11 20°C and CH11 16°C show lower diacetyl levels

**Blend Roussanne & Marsanne**

**Flavor quotation frequency (In senso veritas, april 2012)**

- No citrate uptake → No citrate degradation into diacetyl and acetoin
- No buttery flavor

From publication “Revue des Oenologues, N°161 special, Nov 2016, pp 65-68”
### Viniflora® range effectiveness

<table>
<thead>
<tr>
<th>protection</th>
<th>PRELUDE™</th>
<th>FrootZen™</th>
<th>CONCERTO™</th>
<th>CiNe™</th>
<th><strong>Viniflora® Oenococcus oeni</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Broad protection against yeasts and molds</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specific protection towards yeast <em>Brettanomyces</em></td>
<td>(+)</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Specific protection towards mold <em>Botrytis</em></td>
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<tr>
<td>Protection towards acetic bacteria (<em>Acetobacter</em>, <em>Gluconobacter</em>)</td>
<td>(+)</td>
<td>+</td>
<td>(+)</td>
<td>(+)</td>
<td></td>
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<tr>
<td>Protection towards lactic bacteria (<em>Pediococcus</em>, <em>Lactobacillus</em>, <em>Leuconostoc</em>, <em>Oenococcus...</em>)</td>
<td>(+)</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Anti-oxidation</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Reduction of volatile acidity</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Increase of flavor intensity</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Increase of Freshness/Fruitiness</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>No production of biogenic amines*</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*: Histamine, Tyramine, Cadaverine, Putrescine. (): need to be further investigated
Keep it great!

Improve your bottom line

Use Viniflora® bioprotective cultures to help increase your profitability by getting the best out of the grapes.
Increase your profitability

- **Up-grade musts from low quality grapes**
  Viniflora® cultures both pre-fermentation yeast, Saccharomyces and malolactic bacteria enable bio-control of the whole wine-making process and consistently get the best of the grapes, even in bad years with poor quality of harvest.

- **Increase vintage reliability**
  Year to year variations are reduced with a more reliable wine-making process

- **Get more revenues per hectoliters or per bottle**
  Communicate on your initiatives and convince by tasting to get a good positioning for your negotiations

Highlight: Through the “Wine Value Calculator” tool, we can help to evaluate the net added value to your winery according to your own set of production parameters.
Keep it great!

Conclusion
Recap. on Viniflora® best bio-protective cultures...for safe, healthy, tasty and authentic wines

But also ... Prelude™

Pre-fermentation yeast

Concerto™

FrootZen™

CiNe™

CH16

But also ... Oenos, CH11

Oenococcus oeni

MLF bacteria
Keep it great! with bioprotection

Thank you for your attention