Brettanomyces update

CCOVI Seminar

Brock University

March 8th 2012

Brettanomyces : a global issue for enologists, winemakers &… consumers!

Tiziana Nardi, PhD

Lallemand Italia

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Taking the Barnyard Out of Your Wine

Scientists are decoding the Brettanomyces DNA to stop wine spoilage

Stuart Fox, *Posted: August 7, 2009*

WINE SPECTATOR

'The enemy' at bay

Scientists crack Brett gene code

Adam Lechmere, *Posted:* Monday 28 November 2011

DECANTER

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"The tasting note every winemaker fears. Brett can spoil wines with sweaty horse, barnyard, Band-Aid or burnt plastic aromas and flavors."

Pourquoi le vin a parfois une haleine de cheval?

20/02/2010, émission radio « Les Savanturiers », interview de Pr. Hervé Alexandre, IUVV

FRANCE INTER

www.bourgogne-live.com

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International Wine Challenge, London
Breakdown of Total Faults

(data provided by Sam Harrop MW, personal communication 2010)

	2006	2007	2008	2009	4 yr Av
Total Faults (% of all wines tasted)	**7.1**	**6.9**	**5.9**	**7.3**	**6.8**
Cork Taint	27.8	29.7	31.1	25.7	28.6
Sulphides	29.2	26.5	28.9	25.7	27.6
Oxidation	24.3	22.9	19.1	28.4	23.7
Brett	**10.6**	**12.8**	**13.8**	**15**	**13**
Rot	5.8	5.6	3.4	3.4	4.1
SO_2	1.7	1.8	1.4	0.6	1.9
Other Micro	0.4	0.7	2	1.2	1.1

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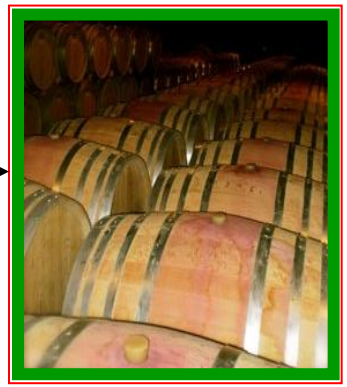
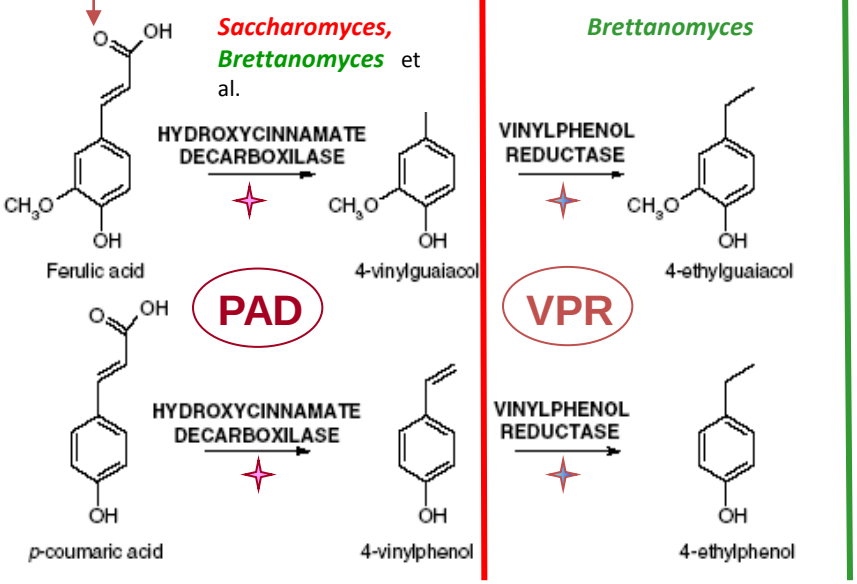
Saccharomyces and *Brettanomyces* in winemaking





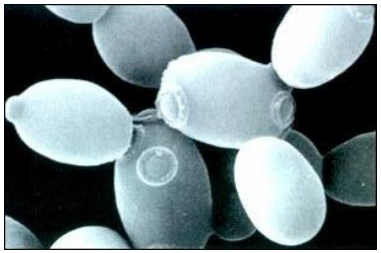
"*Brett*" charachter

mousiness, animal, horsy, barnyard, smoky, spicy, burnt plastic, band-aid, medicinal...

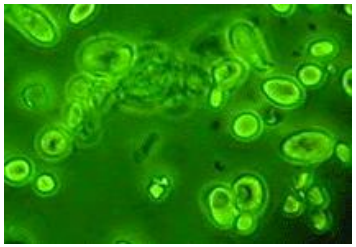
major microbial cause of wine spoilage worldwide

(Heresztyn 1986; Chatonnet et al. 1992; Edlin et al., 1998; Dias et al., 2003; Suàrez et al., 2007; Stratford et al., 2007, Tchobanov et al., 2008)

Brettanomyces bruxellensis



Yeast physiology



Like *S.cerevisiae*:

- Can ferment sugars (Crabtree +)
- Facultative anaerobic metabolism
- Ethanol tolerant (>16%)
- Sulphite resistant

Unlike *S.cerevisiae*:

- Grows slowly at high sugar concentration (non-efficient glucose utilization)
- Can assimilate alternative carbon sources, including ethanol
- SO_2 induces VBNC state

In winemaking:

- *Brett* is very effective in colonizing its habitat: the winery (especially barrels)
- During alcoholic fermentation *S.cerevisiae* dominates
- After AF, at low sugars (300mg/L) and high alcohol, selection favors *Brett*

Coterno et al, 2006; Silva et al., 2004; Agnolucci et al., 2010

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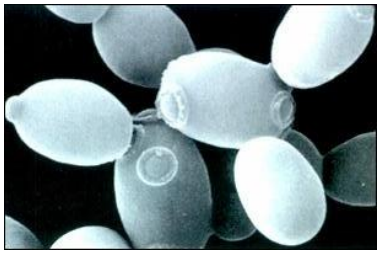
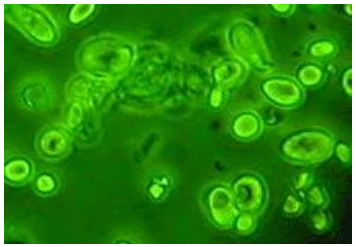
Brettanomyces bruxellensis



Principal cause of microbial spoilage in wine



Molecules produced by *Brett* :

- Acetic acid (volatile acidity up to 2.75 g/L)
  - Tetrahydropyridines (condensation of lysine and ethanol): «mousy smell»
    - Volatile phenols(4-ethyl-gaîacol et 4-ethylphenol) «barnyard and sweaty horse»

Conditions for production are still not completely unveiled:

- A small contamination with small activity can be sufficient
  - 1/6 *Brett* strains do not produce volatile phenols
    - *Brettanomyces* can survive in a Viable But Non Culturable (VBNC) state for long periods.

Heresztyn, 1986 ; Chatonnet et al.,1995 et1997; Ribéreau-Gayon (1998); Millet & Lonvaud-Funel. 2000, Martorell et al., 2006, Conterno et al. 2006, Benito et al., 2010, Serpaggi et al., 2012

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Brettanomyces bruxellensis

Genetics (1)

home › information services › ebulletins › 2011-Nov-23 : current page

eBulletins - 23 November 2011

Wine Researchers Sequence 'Brett' Genome in world first

Scientists at The Australian Wine Research Institute have sequenced the genome of *Dekkera bruxellensis (Brettanomyces)*, the yeast organism commonly known as 'Brett', in a world first for the Australian wine industry.

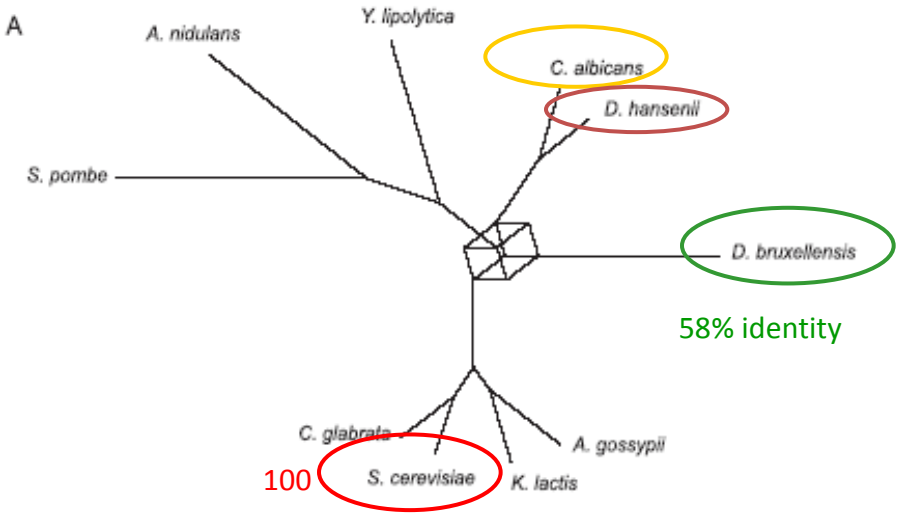
The breakthrough offers Australian winemakers a competitive advantage in managing the troublesome yeast, which has the potential to spoil wine with its 'medicinal' and 'metallic'

"The Australian Wine Research Institute has been working proactively for more than two decades to give Australian wine producers an upper hand against Brett," said Professor Sakkie Pretorius, Managing Director, AWRI.

Brettanomyces bruxellensis

Genetics (2)

- 20 to30 Mb genome
- 40% sequenced in 2007, 100% sequenced in 2011 (assembly not available yet)
- 3000 identified genes (2600 orthologs to *S.cerevisiae*)
- 7400 estimated genes



Woolfit et al., 2007; Hellborg et al., 2009

Among these genes:

- Genes encoding for phenolic acid decarboxylase (**PAD**) and vinyl phenol reductase (**VPR**)
- **Stress response** expected to be partially conserved and partially peculiar
- Genes encoding for entry/exit to **VBNC** status, expected to be peculiar

Kurtzman & Fell, 1998; Conterno et al., 2006, Millet et al., 2000; du Toit et al. 2005, Borneman 2011

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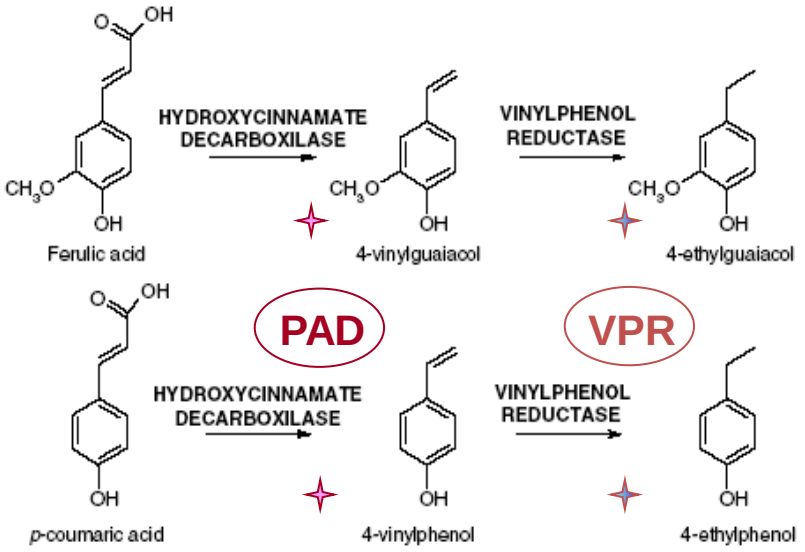
Volatile phenol production

Genes and enzymes

Current knowledge



DOI 10.1007/s00253-008-1708-7

APPLIED MICROBIAL AND CELL PHYSIOLOGY

Survey of enzyme activity responsible for phenolic off-flavour production by *Dekkera* and *Brettanomyces* yeast

Victoria Harris · Christopher M. Ford ·

- Genes are known as well as working conditions for enzymes
- Probably gene expression studies will follow after the genome sequencing

Partial vinylphenol reductase purification and characterization from *Brettanomyces bruxellensis*

Iavor Tchobanov[1], Laurent Gal[2], Michèle Guilloux-Benatier[1], Fabienne Remize[1], Tiziana Nardi[1], Jean Guzzo[1], Virginie Serpaggi[1] & Hervé Alexandre[1]

[1]Institut Universitaire de la Vigne et du Vin Jules Guyot, Université de Bourgogne, Dijon Cedex, France; and [2]ENSBANA, Dijon, France

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Adaptation and stress response (1)

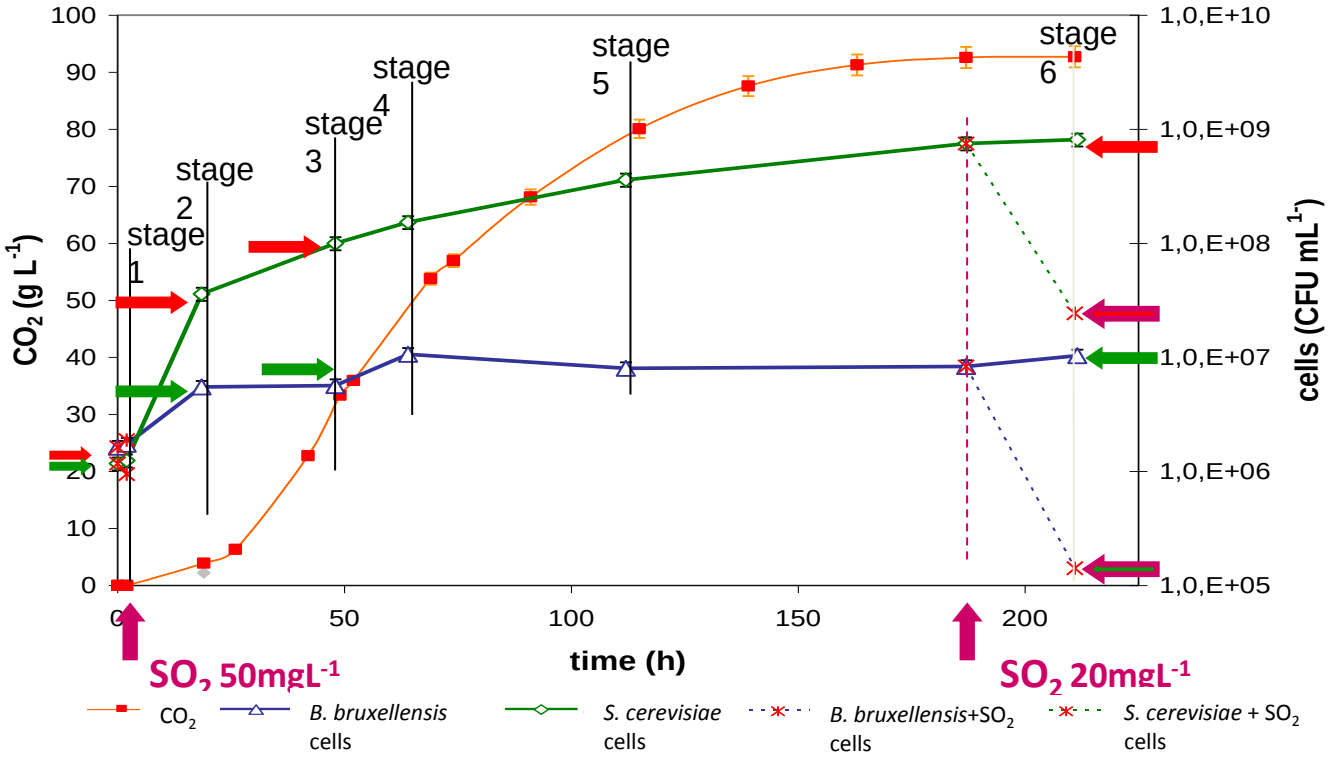
Brett vs *S.cerevisiae* during fermentation

Appl Microbiol Biotechnol
DOI 10.1007/s00253-010-2786-x

GENOMICS, TRANSCRIPTOMICS, PROTEOMICS

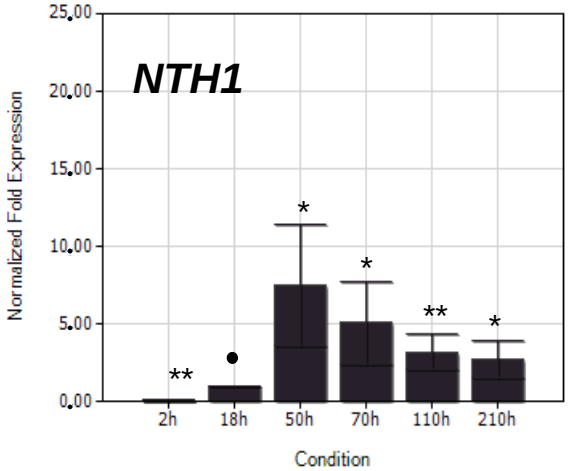
Adaptation of yeasts *Saccharomyces cerevisiae* and *Brettanomyces bruxellensis* to winemaking conditions: a comparative study of stress genes expression

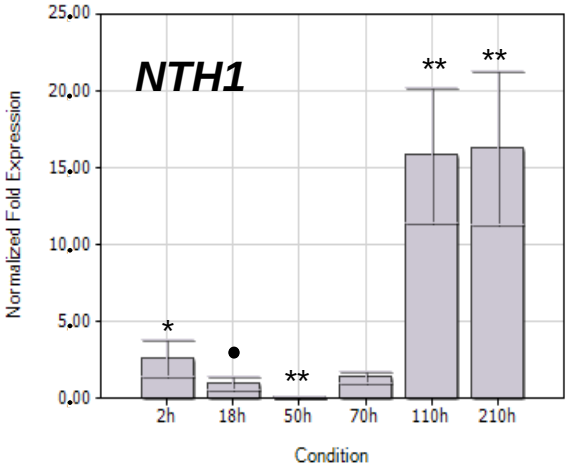
Tiziana Nardi · Fabienne Remize · Hervé Alexandre



Stress response (2) gene expression

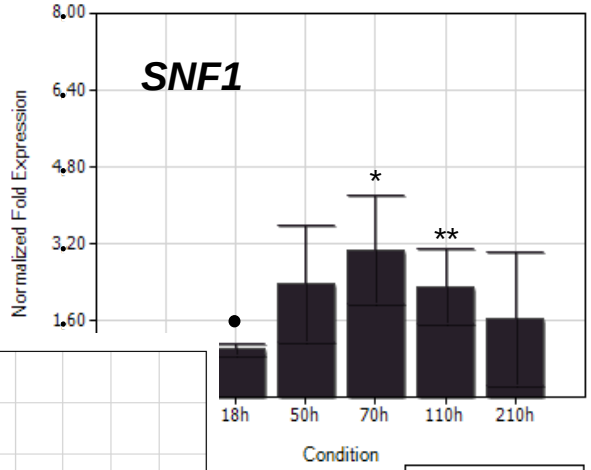
S. cerevisiae | *B. bruxellensis*

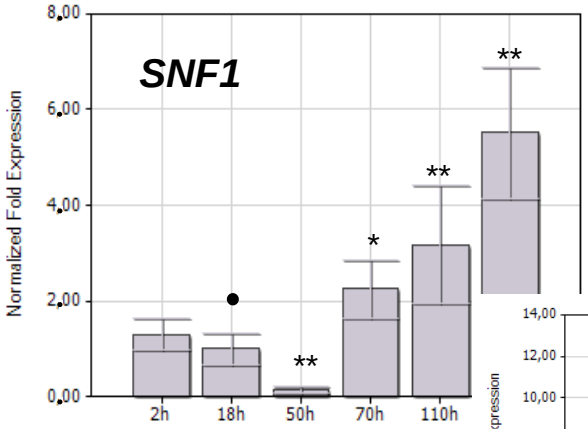




General stress response

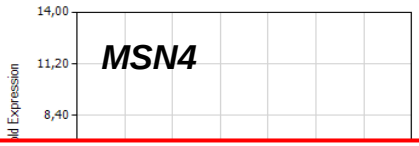
Trehalose futile cycle

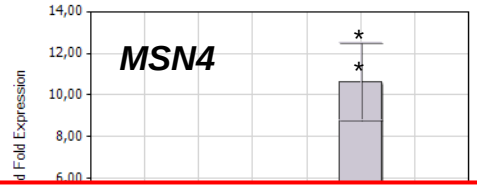




Hsp response

Glucose derepression





Brett stress response seems better fitted for late fermentation stages

Alexandre et al. 2001; Gasch et al., 2000; Causton et al., 2001; Rossignol et al. 2003; Marks et al., 2008, Nardi et al., 2010

VBNC new findings – ongoing studies

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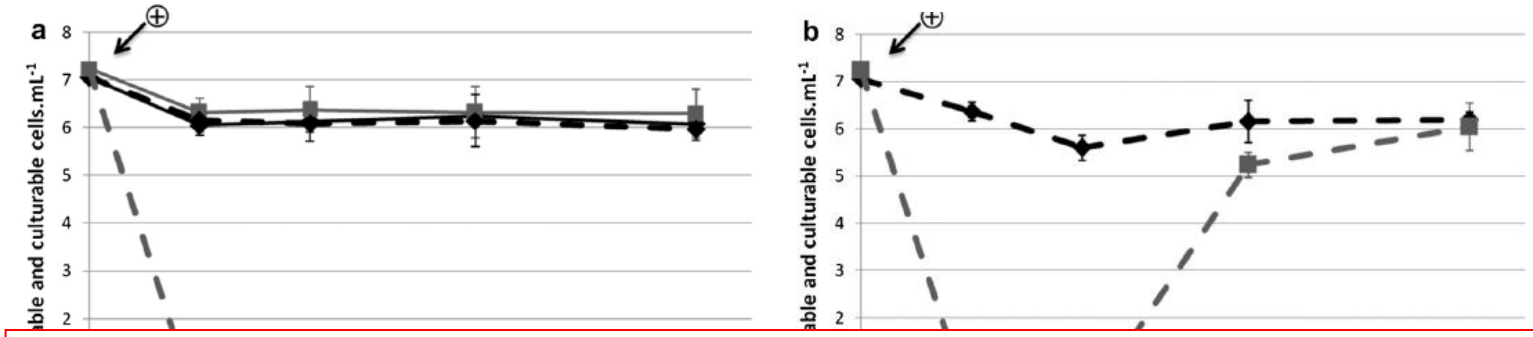
Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Characterization of the "viable but nonculturable" (VBNC) state in the wine spoilage yeast *Brettanomyces*

Virginie Serpaggi [a], Fabienne Remize [a,1], Ghislaine Recorbet [b], Eliane Gaudot-Dumas [b], Anabelle Sequeira-Le Grand [c], Hervé Alexandre [a,*]

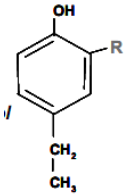
[a] Institut Universitaire de la Vigne et du Vin Jules Guyot, Université de Bourgogne, Rue Claude Ladrey, BP 27877, F-21078 Dijon Cedex, France



- Some protein involved in VBNC have been identified
- Probably gene expression studies will follow after the genome sequencing

Edwards et al., 2007, Serpaggi et al., 2012

How to prevent? (1)



Post-genomic era for *Brett* is coming soon.
… what in the meanwhile?

HYGIENE

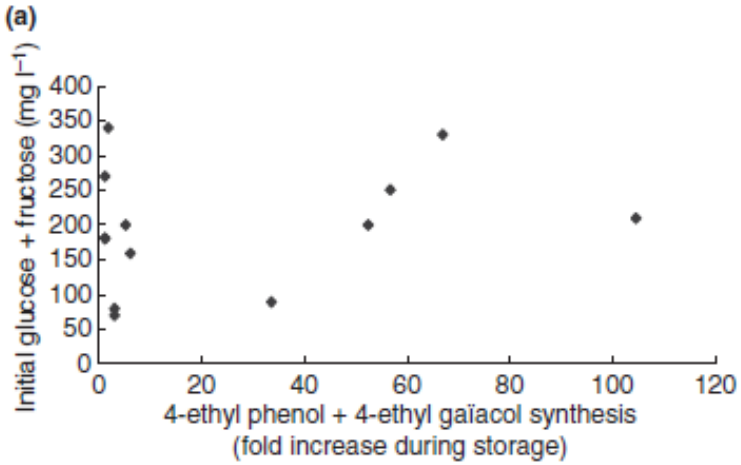
Not only barrels! *Brett* found in different niches in the winery

TIME

- **Alcoholic Fermentation management** - Prevent stuck or sluggish fermentations by using selected yeasts

- **Malolactic Fermentation management** - Prevent late, stuck or sluggish fermentations by using selected bacteria and co-inoculation, stabilise wines earlier

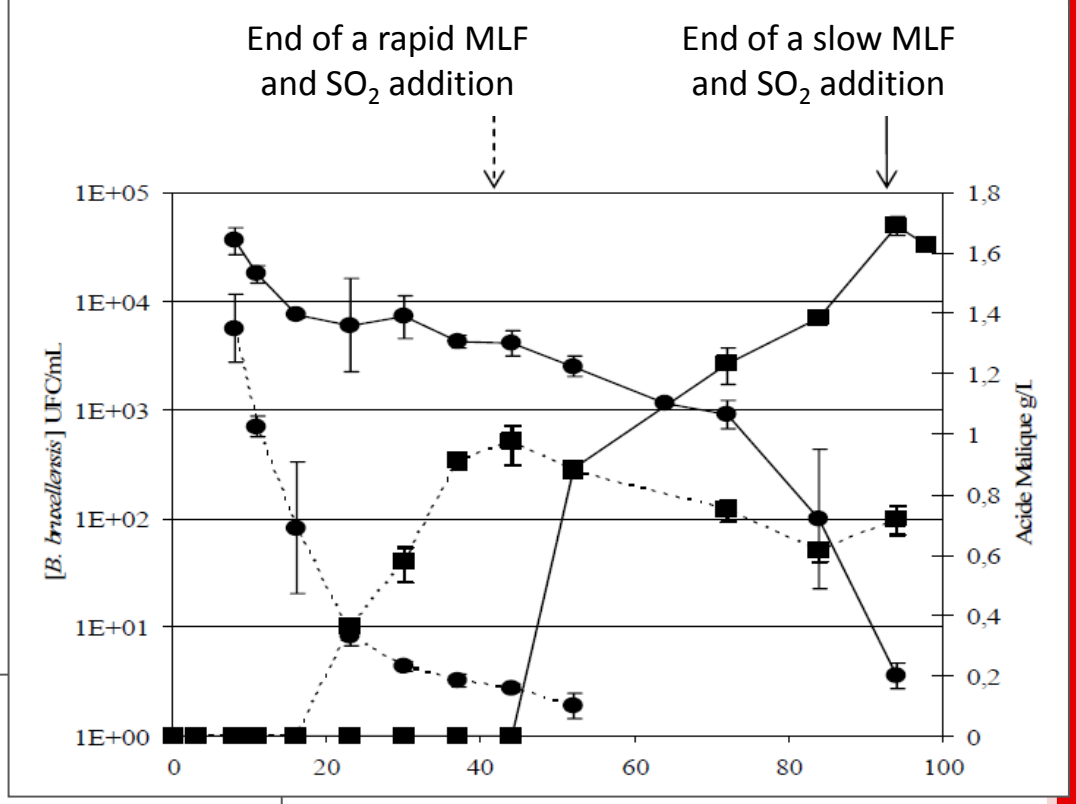
J. Coulon *et al.*



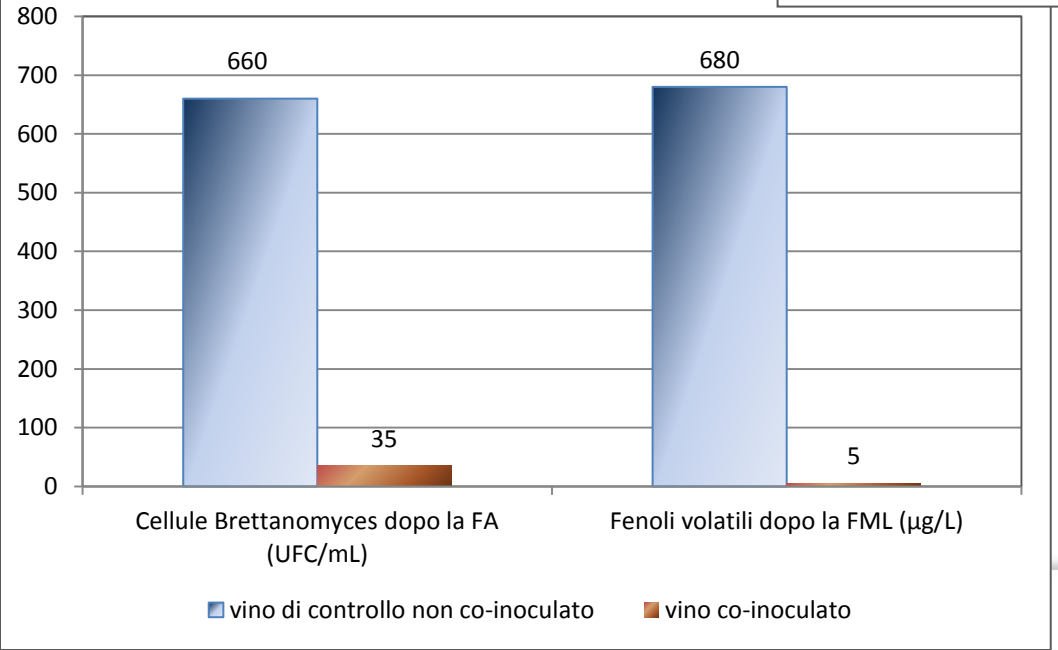
Malherbe et al., 2007; Gerbaux et al. 2009, Coulon et al., 2009

How to prevent? (2)

MLF and SO_2 management



Brettanomyces **population and volatile phenols concentration in Cabernet Franc before and after MLF**



Gerbaux et al. 2009, Renouf et al., 2006

How to prevent (3) when sulphur dioxide is not enough

Short Communication

Sulphur dioxide affects culturability and volatile phenol production by *Brettanomyces/Dekkera bruxellensis*

Monica Agnolucci [a], Francesco Rea [a], Cristiana Sbrana [b], Caterina Cristani [a], Daniela Fracassetti [c], Antonio Tirelli [c], Marco Nuti [a,*]

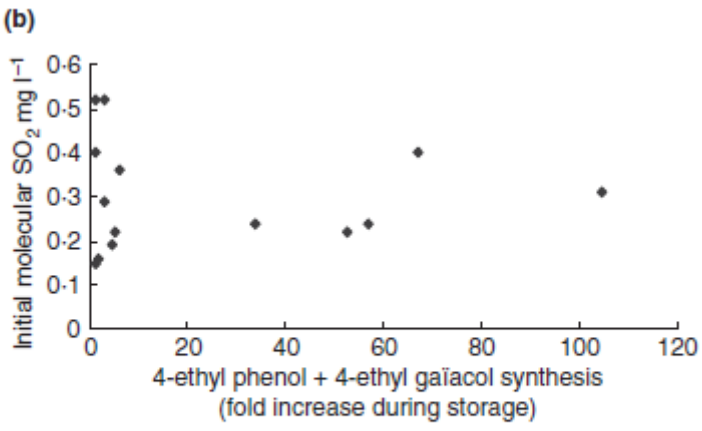
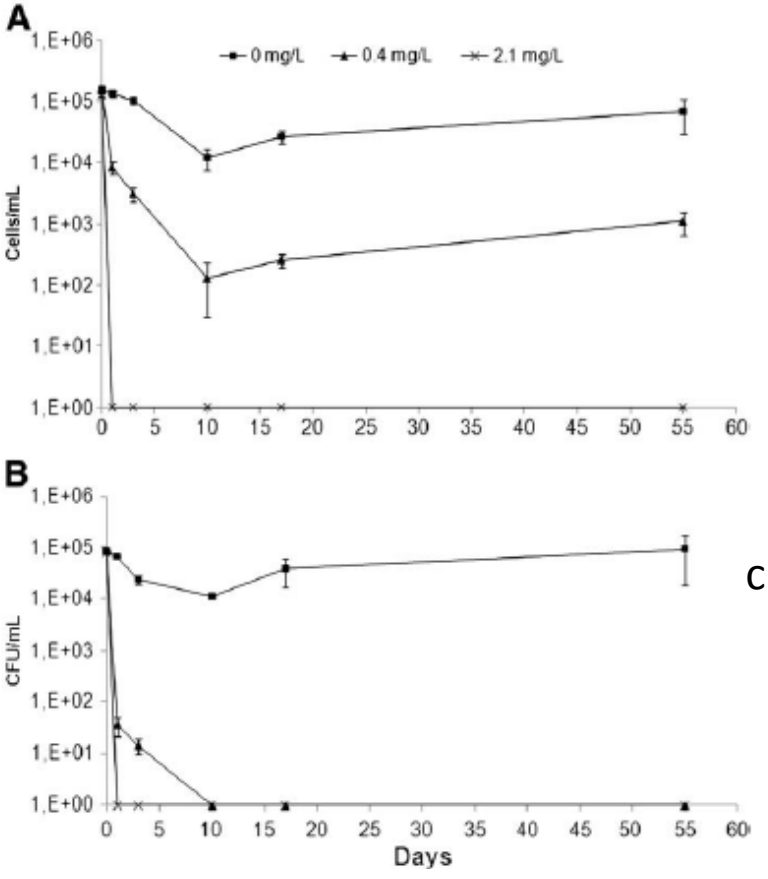


Figure 1 Correlation between ethyl-phenols synthesis and initial glucose + fructose concentration (a) and molecular SO_2 (b).



viable

culturable

Fig. 1. Effect of different molecular SO_2 concentrations on the viability and culturability of *Brettanomyces bruxellensis* 1 L in SWM. (A) number of viable cells/mL, calculated from microscopic cell counts in a Thomas chamber after viability staining with trypan blue; (B) number of CFU/mL, calculated from CFU counts after plating on YPD agar medium. The

Coulon et al., 2009; Agnolucci et al., 2010

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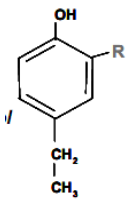
How to solve? (1)



Curative methods towards volatile phenols

- **Common absorbents** used in oenology : PVPP @ 60-480 mg/L and cha. @ 15-240 mg/L were reported to lower ethyl-phenols levels (Sùarez et al., 2007)

- **ADY and yeast lees** (Chassagne et al, 2005) : removal of 33% and 26% of the 4-EP and 4-EG concentrations in a model wine solution.

- **Specific yeast hulls**: R&D project - Lallemand-IUVV collaboration

- **Filtration & adsorption** : ex: process developped by Inter-Rhône / Bücher-Vaslin >> elimination of more than 90% of volatile phenols (P. Vuchot et al., OIV 2007)

-**Cellulose polymers :** 30% average removal with cellulose acetate and propionate (Nicolini et al., 2011)

How to solve ? (2)

Curative methods towards *Brettanomyces* cells

- **Filtration** : Removal of *Brettanomyces* cells using membranes with a pore size smaller than 0.45 µm (Calderòn et al., 2004)

- **Fining agents** : *Brettanomyces* populations can be reduced by 40 to 2000-fold by treatments with fining proteins (Murat & Dumeau, 2003). *Brettanomyces/Dekkera* yeasts in a red wine have also been decreased from an initial population of 10^4 CFU/mL to 170 CFU/mL after fining with liquid gelatin @ 0.5mL/L (Suàrez et al., 2007).

- Temperature : Couto et al. (2005) showed that a population of 10^6 of *Brettanomyces* could be thermally inactivated with a 37.5°C for 6 minutes, and 41°C for 0.6 min treatment >> **Flash Pasteurization**/heat treatment application development

- **DMDC / Velcorin** : Efficiency of DMDC for the prevention & elimination of Brettanomyces during winemaking @ 200 mg/L (Renouf et al., 2007).
It requires an approved dosing unit and must be carefully handled.

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How to solve ? (4)

Chitosan of fungal origin

A new tool to cope with *Brettanomyces*

NO BRETT INSIDE

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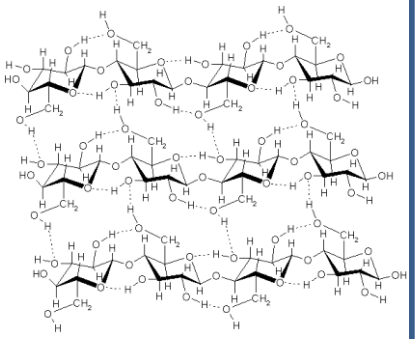
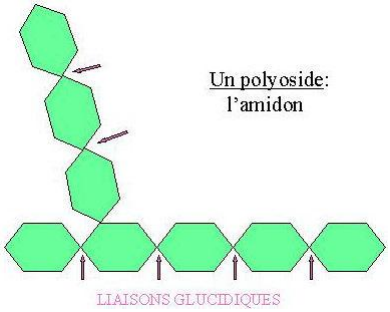
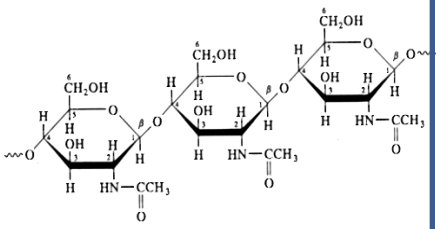
CHITIN & CHITIN DERIVATES

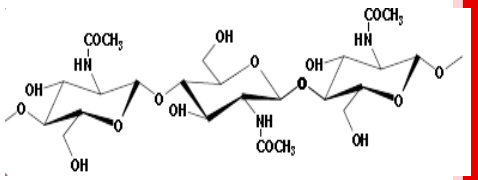
Chitin, **chitin-glucan**, **chitosane**, **hydrolysed chitine-glucan** are part of the big family of natural polymers such as cellulose, starch, collagen etc….

Aspergillus niger





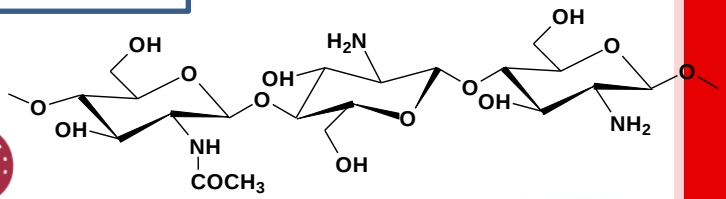




Chitin

Desacetylation

Chitosan



NO BRETT INSIDE

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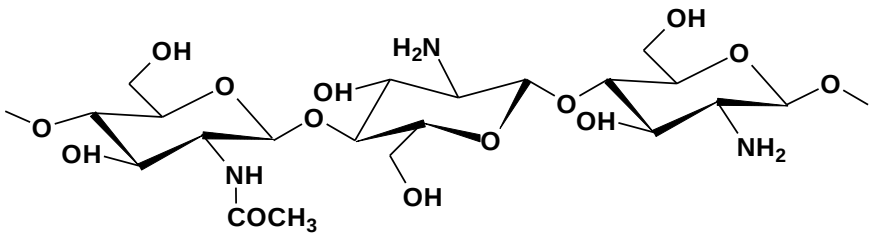
Wide application range of Chitosan



Chitosan

Health

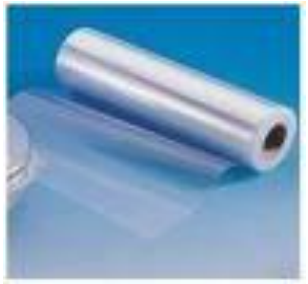
- Opthalmology
- Medical
- Surgery

Agriculture

- Plant care

Cosmetics

- Deodorant
- Toothpaste

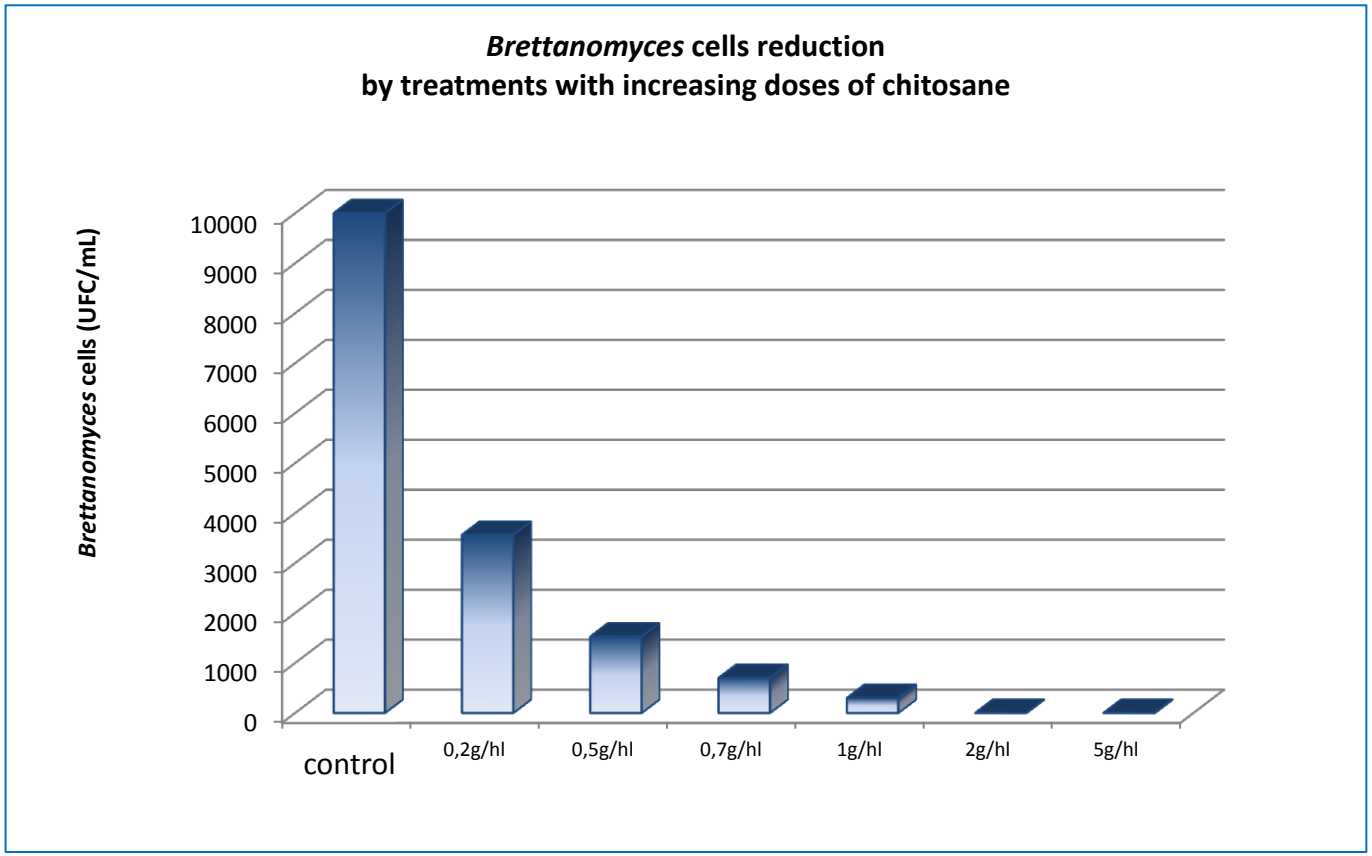


Food

- Preservation of food
- Active packaging
- treatment of waste water

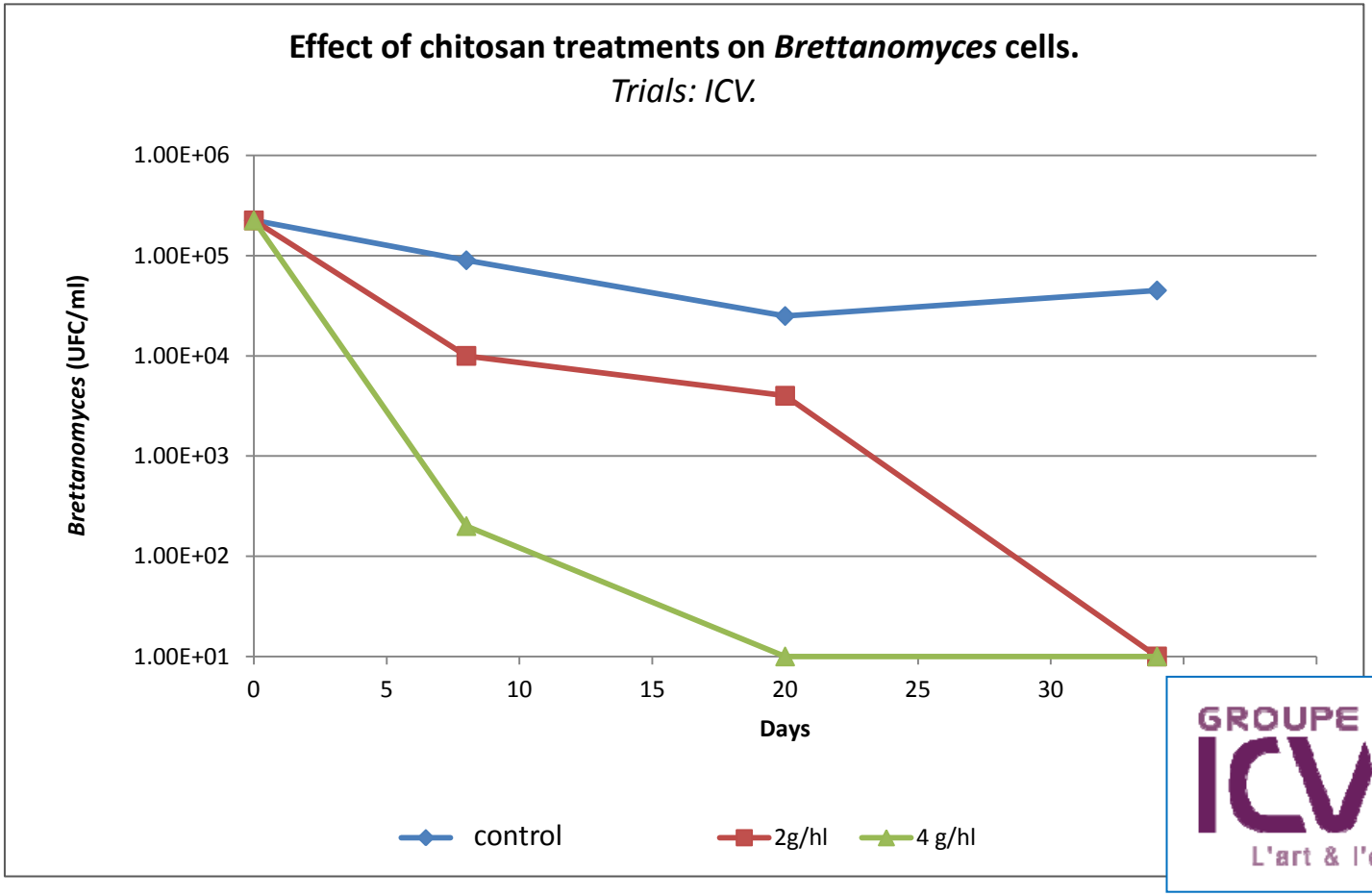
Oenology

Chitosan as a new tool in the struggle against *Brettanomyces*



From OIV Proceedings, 2008, A. Bornet & PL Teisseidre (Fac. d'Oenol. Bordeaux), Elimination des goûts terreux (la géosmine) et des Brettanomyces par l'utilisation d'un biopolymère fongique : le chitosane

Chitosan treatments
Dose effect



GROUPE ICV
L'art & l'expertise du vin

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Chitosan treatments
Dose effect & quantification methods

Trial on a 2009 Burgundy wine, naturally contaminated with *Brettanomyces* (3,7 10^6 cells/mL). Quantification by RT-PCR and counting on semi-selective medium plates of *Brettanomyces bruxellensis* 10 days after treatment with chitosane @ different dosages. No treatment on control.

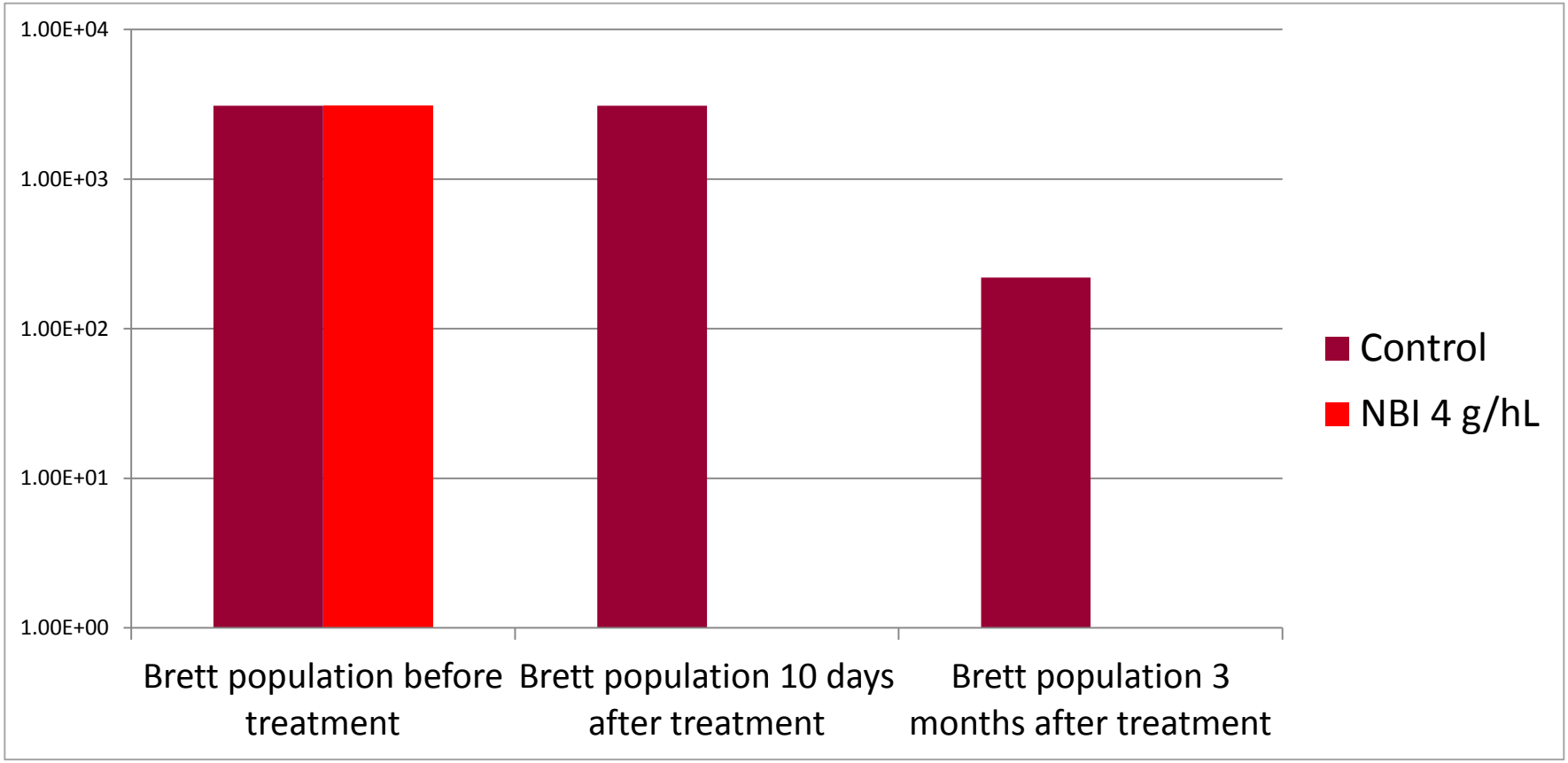
Dosage of treatment	Control	0 g/hl	2 g/hl	3 g/hl	4 g/hl
Quantification of *Brettanomyces* (RT-PCR) (UFC/ml)	3.7×10^6	8.8×10^6	2.3×10^2	1.0×10^3	30
Counting on plates *Brettanomyces* (semi-selective medium) (UFC/ml)	1.1×10^6	1.8×10^6	30	<1	1

Chitosan application trials - Spain 2011,

Collaboration with Excell Iberica

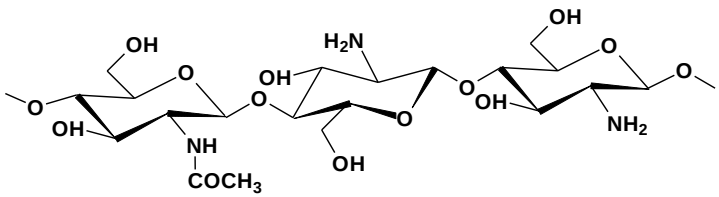
Winery-scale trial, Bodega CVR

Brettanomyces population (UFC/mL), RT-PCR



ICV trials: Duo-trio tasting results show that either there's no difference between the control and the treated wine, or when there's one, the treated wine is prefered.

Action mode of CHITOSAN on *Brettanomyces* ? (1)



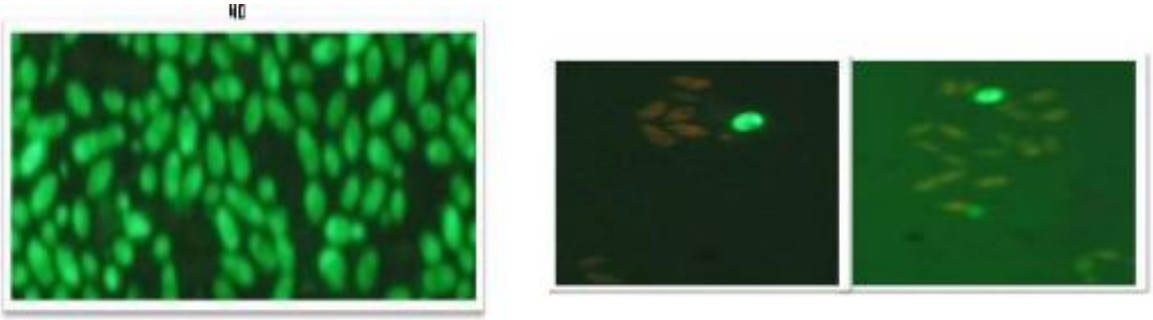
Major hypothesis :

1/ Interactions between Chitosan – cell-membrane >
Transcritional response mechanism, type stress reaction >
disrupting of cell membrane organization inducing a
gene expression response

possibly this will be better known in the next future thanks to new genetic info on *Brett !*

2/ Adsorption on cell-wall & blocking of transfers
between intra- and extra-cellular media

Action mode of CHITOSAN on *Brettanomyces* ? (2)



Control sample (non diluted)

Samples treated with chitosan, 4 g/hL (diluted 1/10)

Figure 1 : Cells of *Brettanomyces* with epifluorescence microscopy (X1000) in the lees 10 days after treatment. Live cells are colored in green with the CFDA and dead cells are colored in red with IP.

		Counting RT-PCR (UFC/ml)	Counting on plate (UFC/ml)
Bordeaux 2010	Before treatment	12 000	/
	10 days after treatment	5000	<10
	20 days after treatment	3300	< 10
	30 days after treatment	< 10	< 10

Table 1 : Folllow-up of the *Brettanomyces* population on a wine treated with chitosan (4 g/hL). Comparison of counting on plate and RT-PCR datas.

Epifluorescence microscopy, plate counting and RT-PCR analysis results seem to show that **chitosan induces a sub-lethal state** to the *Brettanomyces cells,* prior to their death.

Blayteron et al., ASEV poster, 2011

A NEW TOOL TO CONTROL *BRETTANOMYCES*

TREATMENT	+	-
FINING	Cost Soft treatment	Non specific Low efficiency
FLASH-PASTEURIZATION	Efficiency Quick / instantaneous	Cost Requires equipment « Tough » treatment
DMDC	Quick / instantaneous Efficiency	Cost Requires equipment For some countries, regulatory limits *
Chitosan	**« Soft » treatment** **Biotechnological** **Health & Environment friendly** **Efficient and easy to use**	**Time of action (10 days)** **Cost**

* **EU:** DMDC is authorized by the OIV only for the treatment of wines containing more than 5 g/L of sugars in a short delay before bottling. Authorized in the USA.

LALLEMAND

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Thanks for your attention !