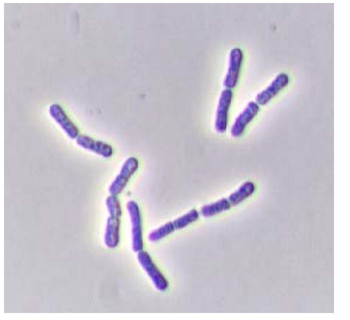
Malolactic fermentation 2005

- R-e-s-p-e-c-t
  the bacteria in your wines
  and
  manage your MLF

Geneva on the Lake, February, 2005

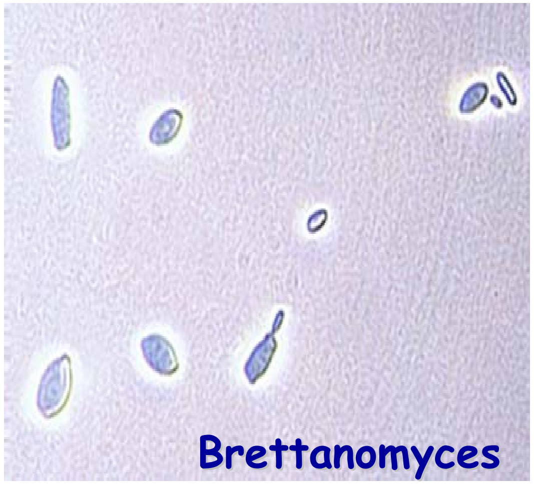
BACTERIA IN MUST & WINE

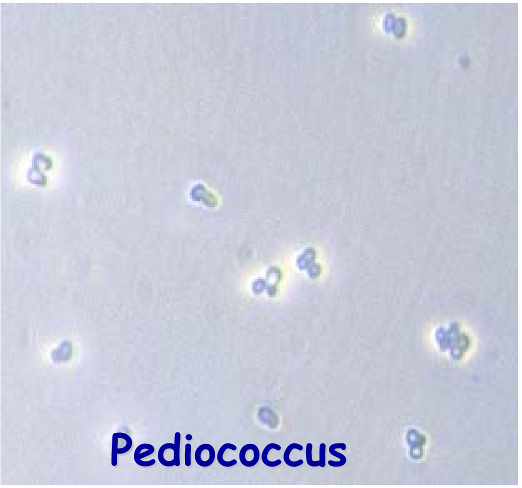


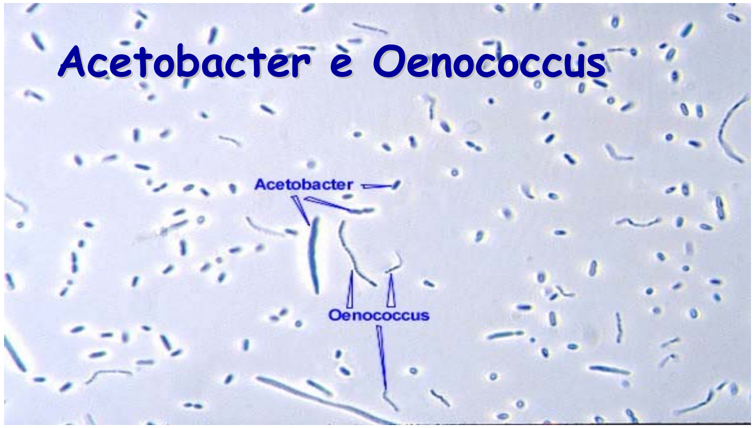
- *Only a few bacteria are adapted to survive in wine*

alcohol
acidity
Low pH???
SO_2
Nutrient deficiency

Microflora in must and wine







THE CHEMISTRY...

$$HOOC \cdot CH_2 \cdot CHOH \cdot COOH \longrightarrow CO_2 + CH_3 \cdot CHOH \cdot COOH$$

malic acid	carbon dioxide	lactic acid
134	44	90

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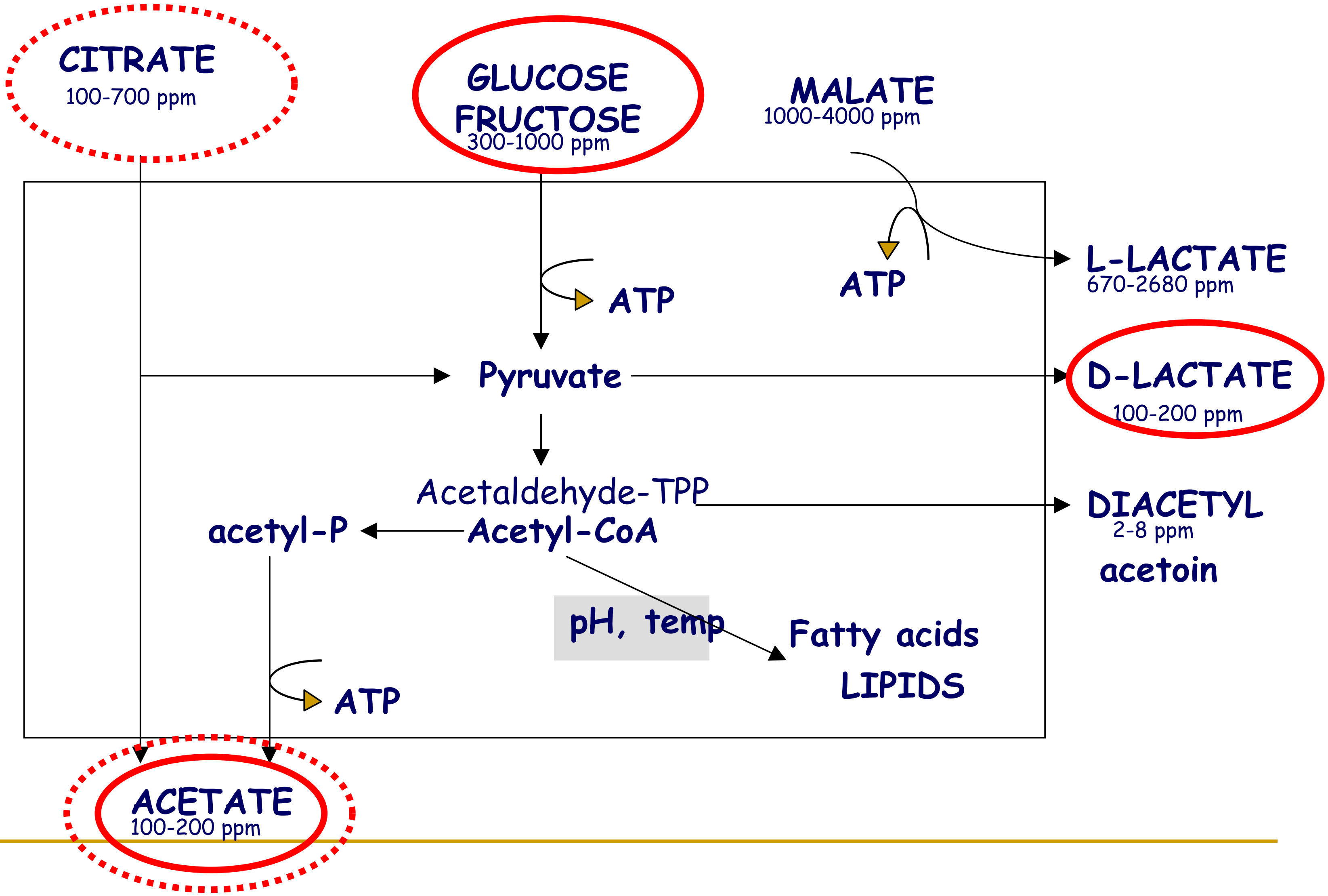
THE CHEMISTRY

- L (-) malic acid converted into L(+) lactic acid (commercial additions of D(+) will remain untouched).
- Not really a ‘fermentation’ as no energy is produced
- Reduction of acidity by 1-3 g/L
- Addition of diacetyl: good or bad?

Metabolism in heterofermentative Lactic Acid Bacteria



Bacteria found in must and in wine

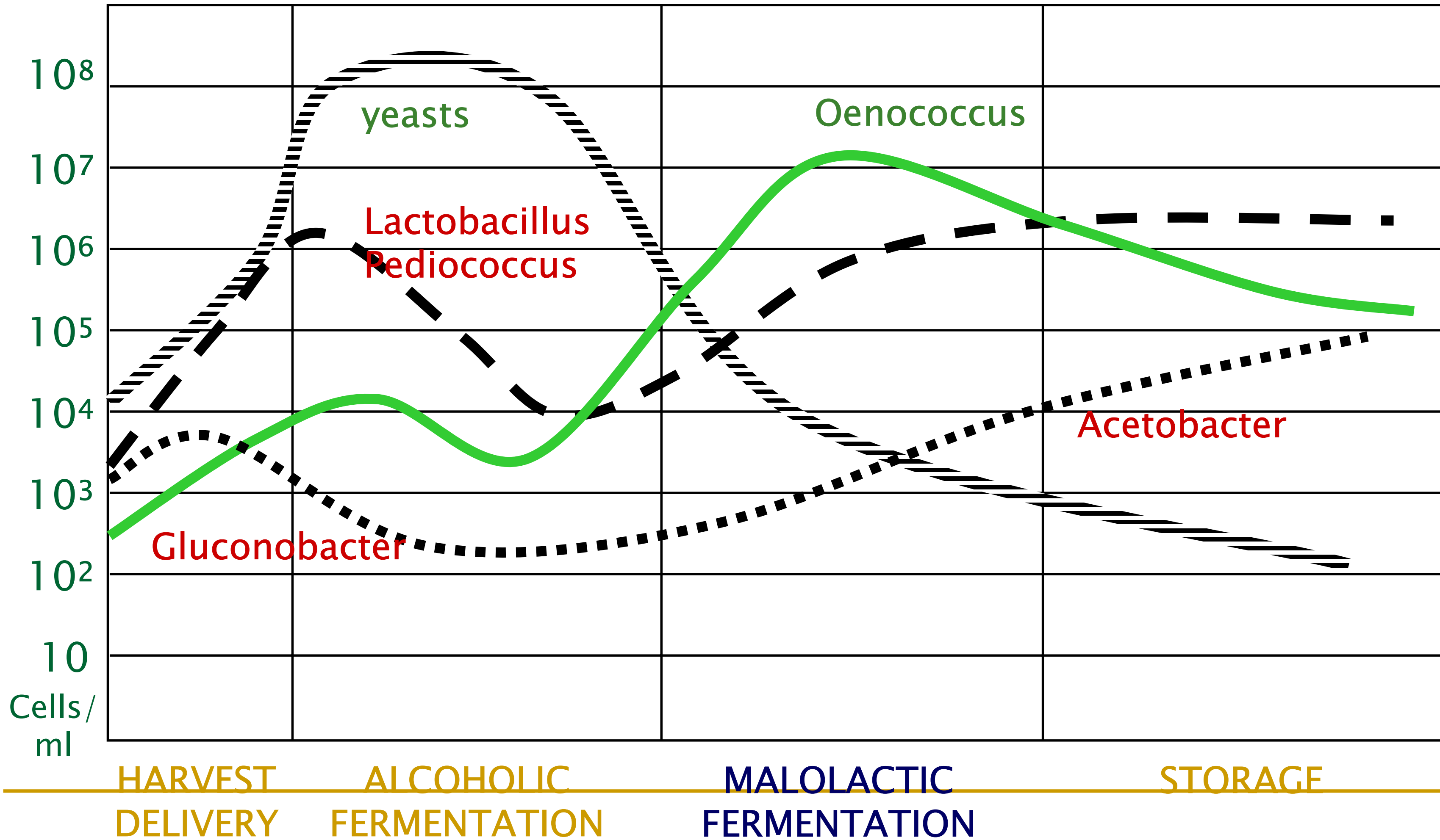
LACTIC ACID BACTERIA

	fermentation:
***Oenococcus oeni* (ex *Leuc. oenos*)**	**hetero**
Leuconostoc mesenteroides	**hetero**
Lactobacillus plantarum	**homo**
Lactobacillus casei	**homo**
Lactobacillus brevis	**hetero**
Pediococcus damnosus	**homo**
Pediococcus pentosaceus	**homo**

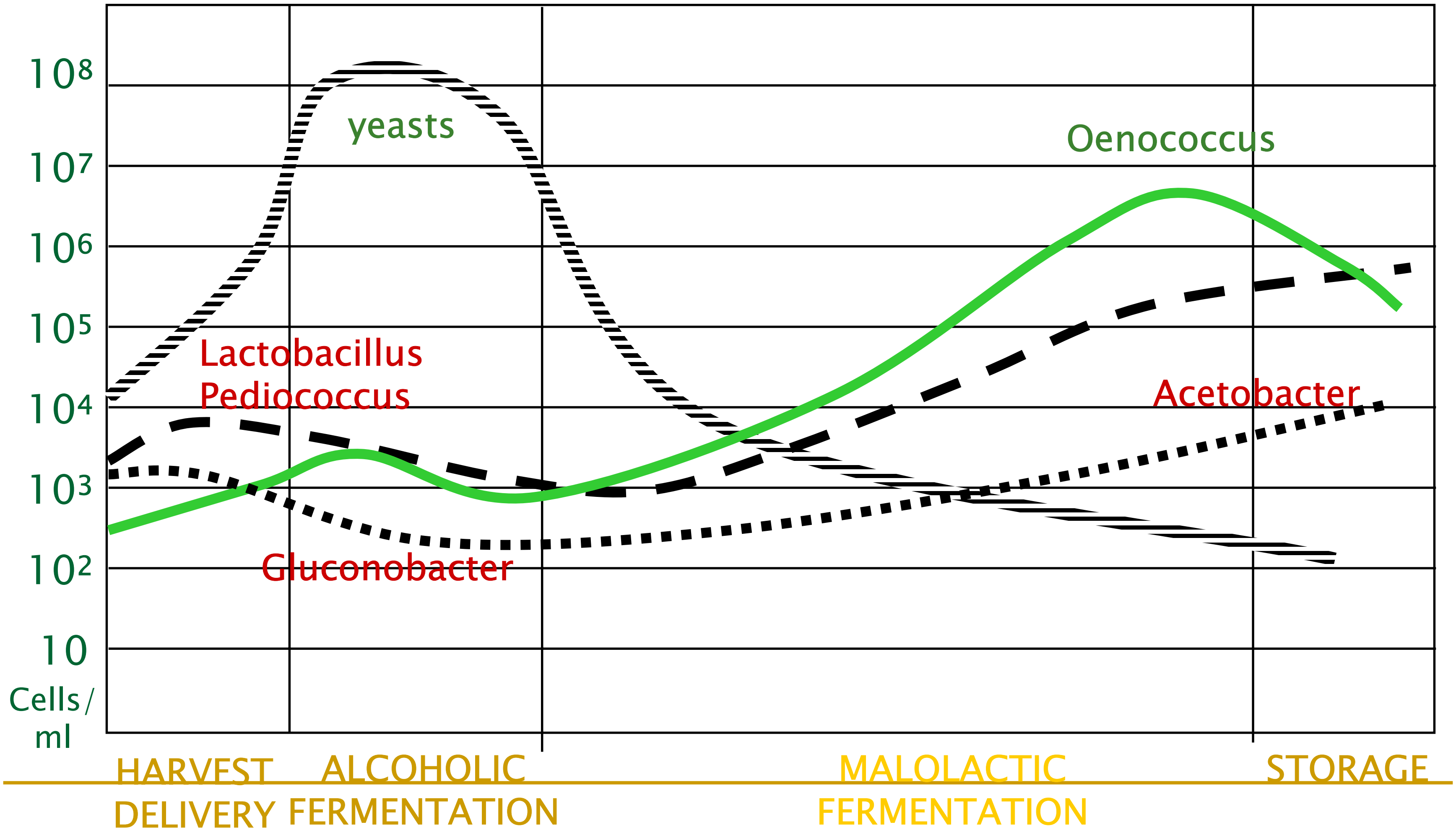
ACETIC BACTERIA

Gluconobacter oxydans	**sugars**
Acetobacter aceti	**ethanol**
Acetobacter pasteurianus	**ethanol**

BACTERIA EVOLUTION
UNDER FAVOURABLE CONDITIONS



BACTERIA EVOLUTION UNDER DIFFICULT CONDITIONS



Most important parameters

CHEMICAL/PHYSICAL

SO_2 > pH > temperature > alcohol

NUTRIENTS

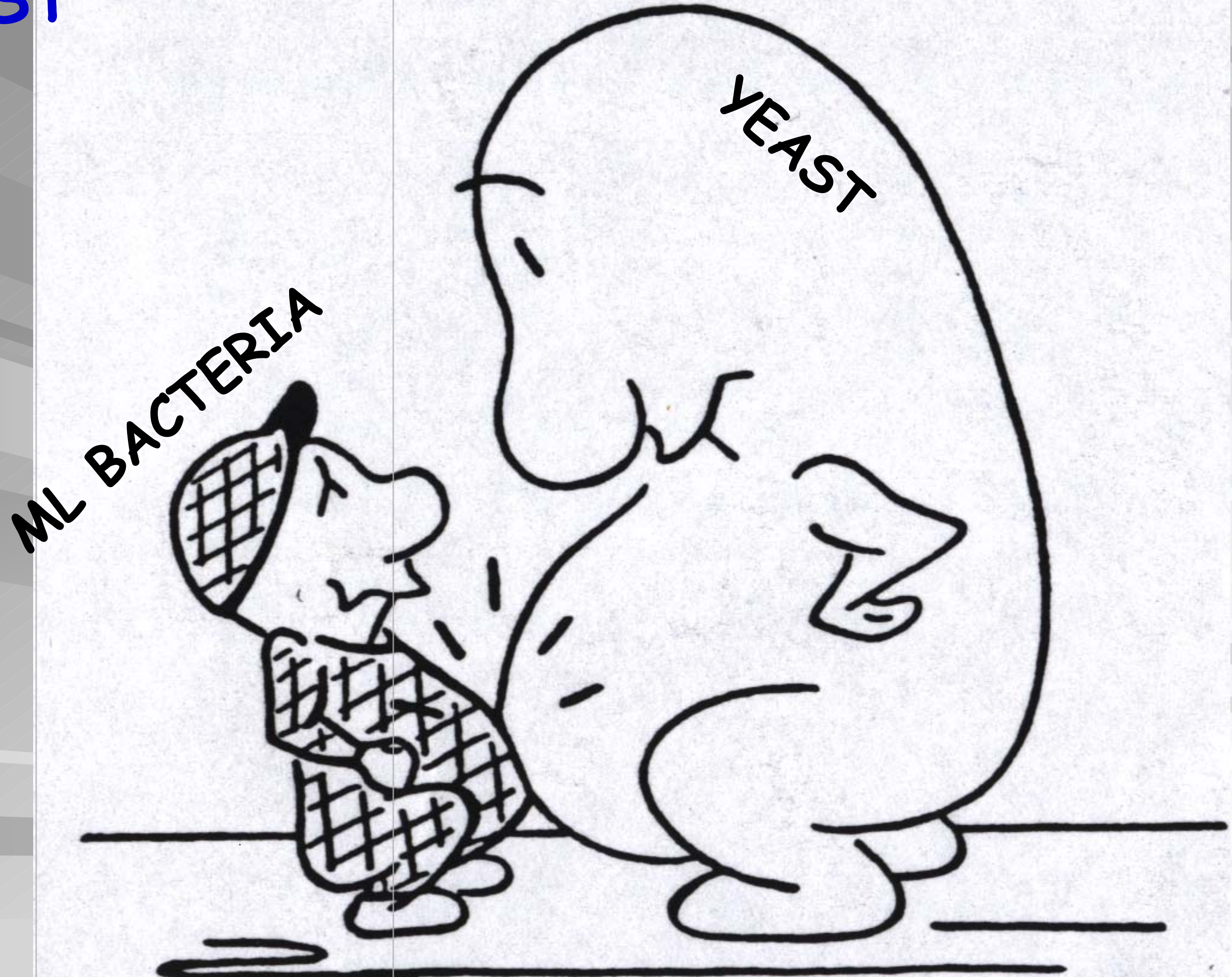
(achtung! O. Oeni cannot use ammonia)

MICROBIOLOGICAL

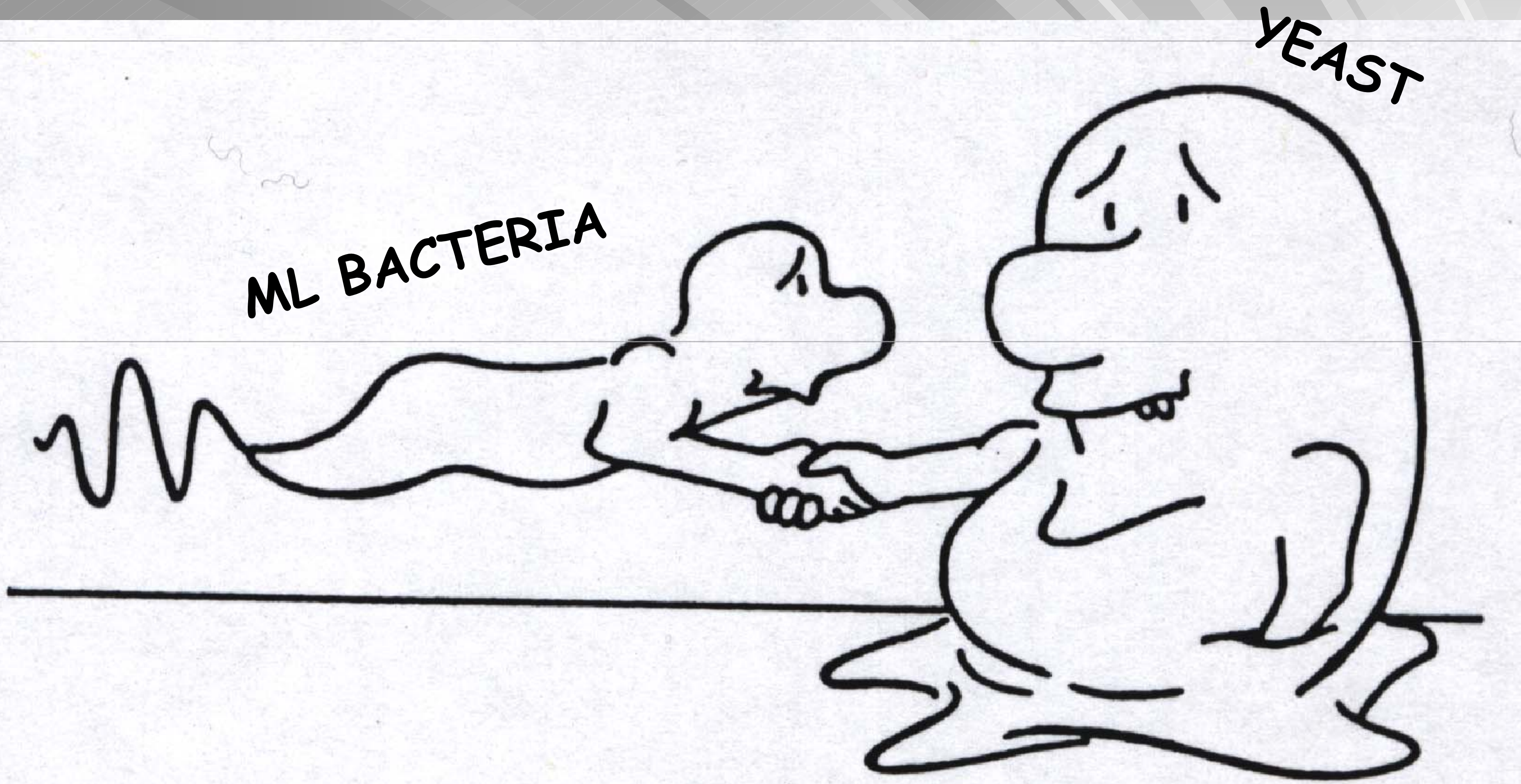
Influence of the wine yeast from AF

POSSIBLE INTERACTIONS BETWEEN YEAST & ML BACTERIA

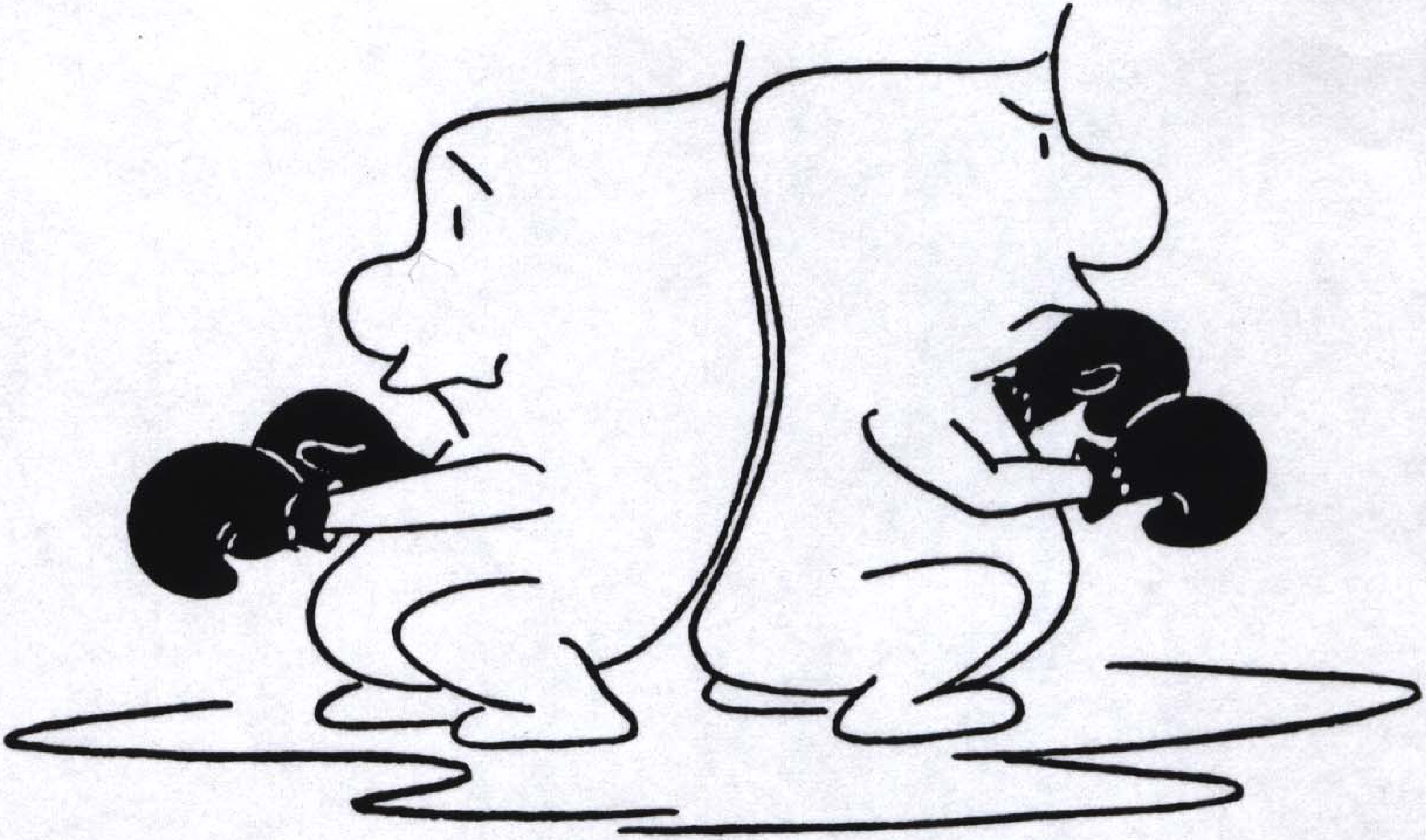
INHIBITION OF OENOCOCCUS OENI BY THE YEAST



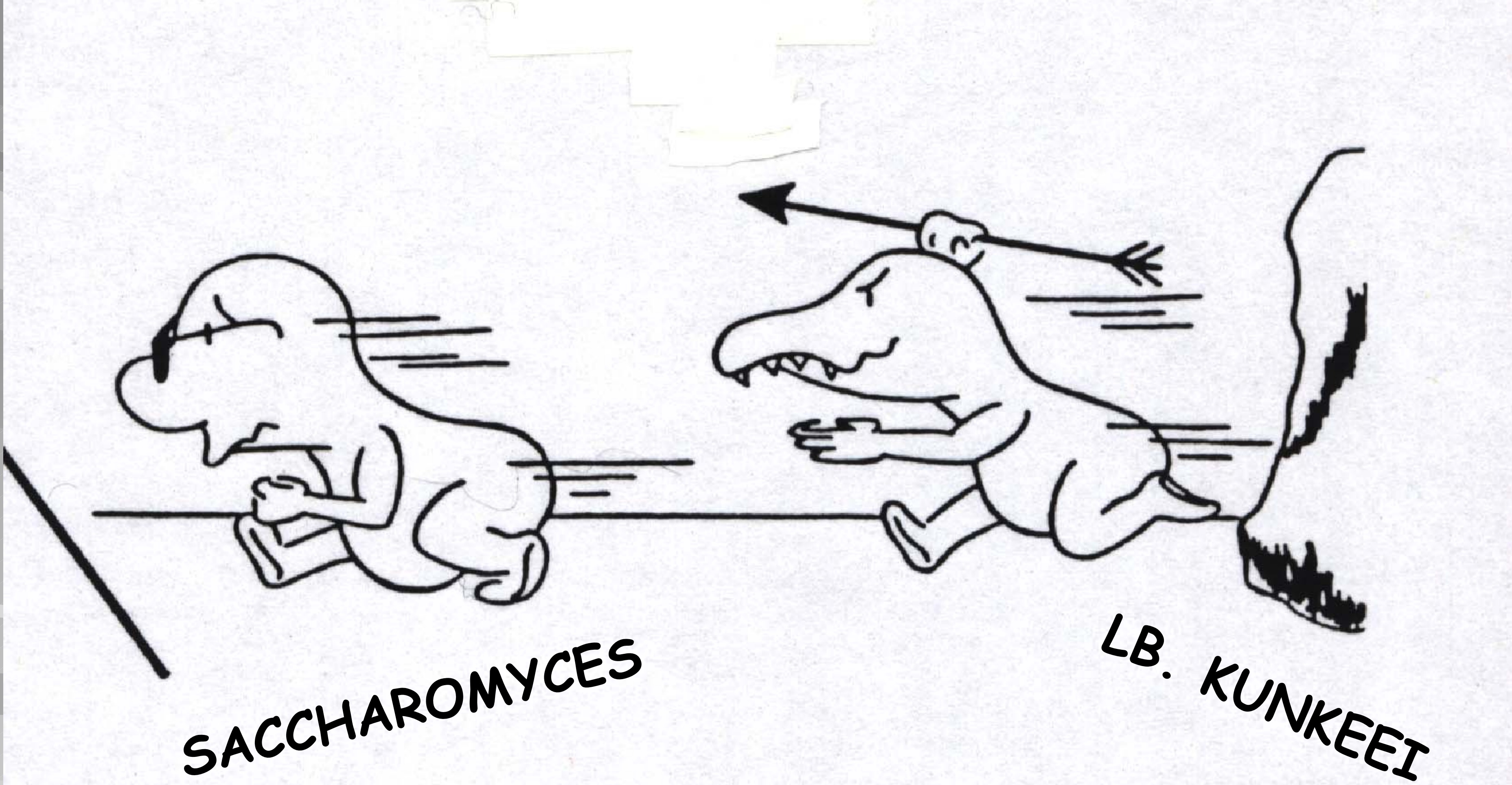
STIMULATION OF OENOCOCCUS OENI BY THE YEAST



NO INFLUENCE = INDIFFERENT



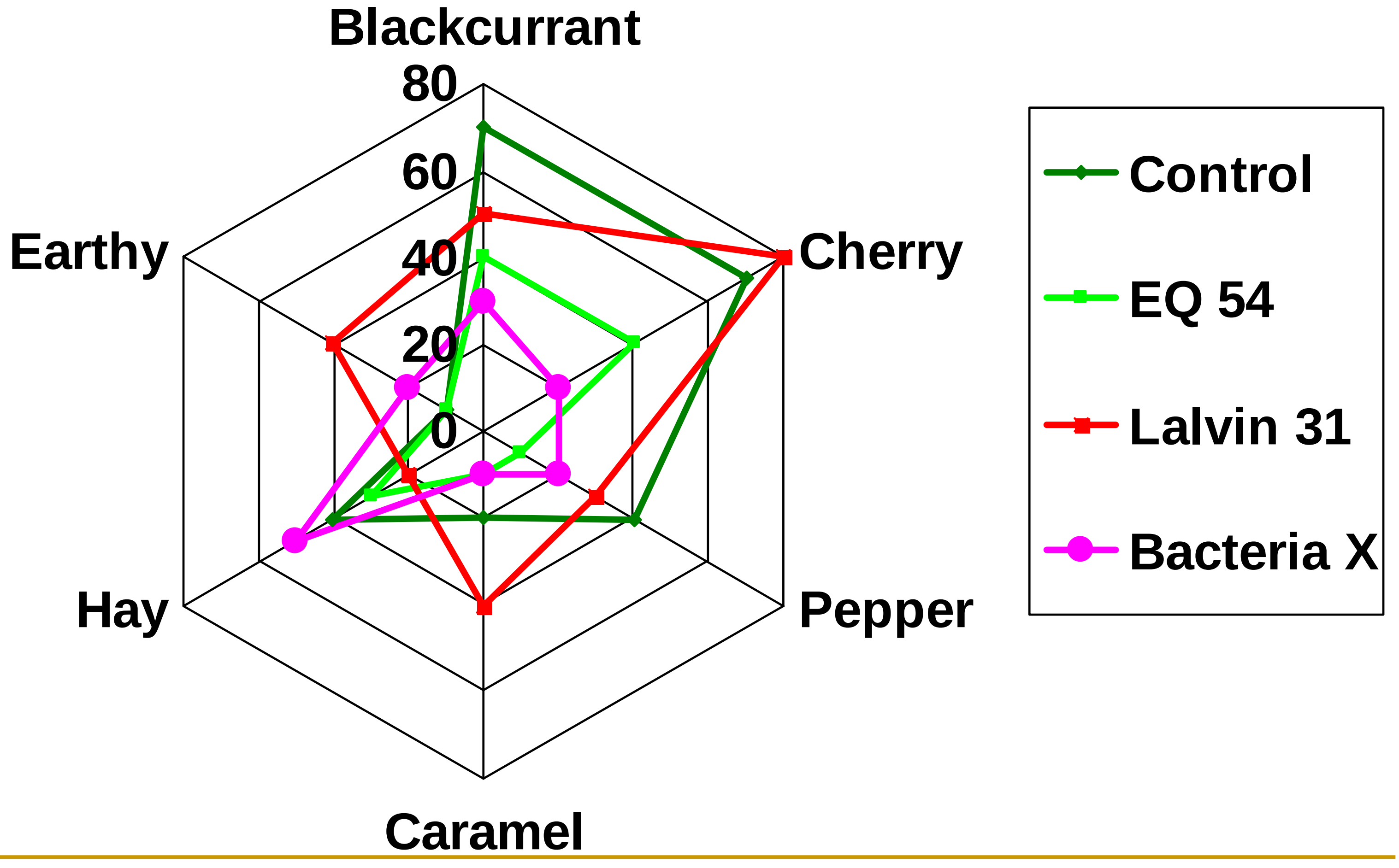
IN SOME CASES INHIBITION OF SACCHAROMYCES CEREVISAE BY WILD LACTIC ACID BACTERIA WAS REPORTED



IN SOME CASES THEY EVEN CAN HAVE MUCH FUN

ITV 99 - Tasting

Pinot noir – Qualitative differences



ML Properties based on organoleptic properties

Maintain fruit characterisitcs

Reduction of vegetal aromas

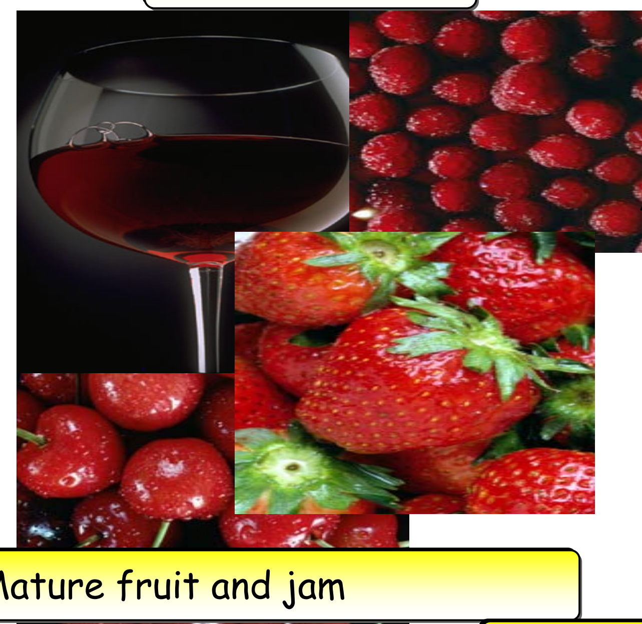
WHITE WINES

-tropical fruit,
-vanilla in barrel fermentation
-peach and melon characterisitcs

Heightened

MOUTHFEEL: volume and balance in mouth

RED WINES



Mature fruit and jam

Increase

Uncontrolled Malolactic Fermentation...

THE MASKS...

(MLF Sensory Defects Kit) presented by...

Dr. Sibylle Krieger
Didier Theodore
Dr. Antonio Palacios

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Winemakers are concerned with:

•Limiting chemical inputs (optimizing the SO_2 dosage).

•Limiting health risk concerns and spoilage (low biogenic amines).

•Avoiding heavy curative treatments of clarification, filtration y stabilization.

•Developing and stabilizing positive aromas tannin perception.

•Maintaining quality through the winemaking process

Wine bacteria: The drawbacks...

- •Volatile Acidity
- •Too much Diacetyl
- •Undesirable Aromas & Flavors
- •Varietal character loss
- •Color loss
- •Ethyl Carbamate
- •Biogenic Amines
- •Geranium Aromas

The Usual Suspects:

Some *Oenococcus*

Many *Lactobacillus*

Many *Pediococcus*

Wine Bacteria: The positive side…

- Lowering acidity
- Ethyl Lactate / Diacetyl
- Varietal aroma enhancement
- Reducing Vegetative notes
- Rounding the mouthfeel
- Lowering astringency
- Lowering bitterness
- Increasing complexity
- Lowering overall SO_2

The Usual Suspects:
Some *Oenococcus*

Ethyl Lactate

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Ethyl Lactate Aroma

- ➔ Formed mainly during MLF.
- ➔ Low levels contribute mouthfeel volume.
- ➔ High concentrations add milk and yogurt aromas.
- ➔ Above 15 mg/L is usually considered negative in wines.

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Acetaldehyde

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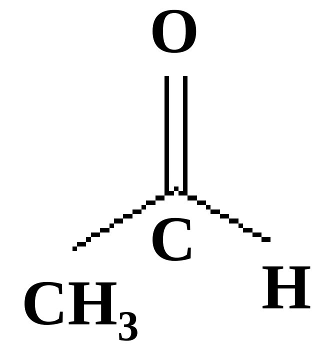
ACETALDEHYDE



- Threshold detection ~ 100 mg/L.
- Odor: overripe apple, fish in vinegar, “oxidation”.
- Sometimes formed during MLF, and other times its levels are reduced.
- Normaly the levels are reduced by binding with free SO_2.

Geranium Odor

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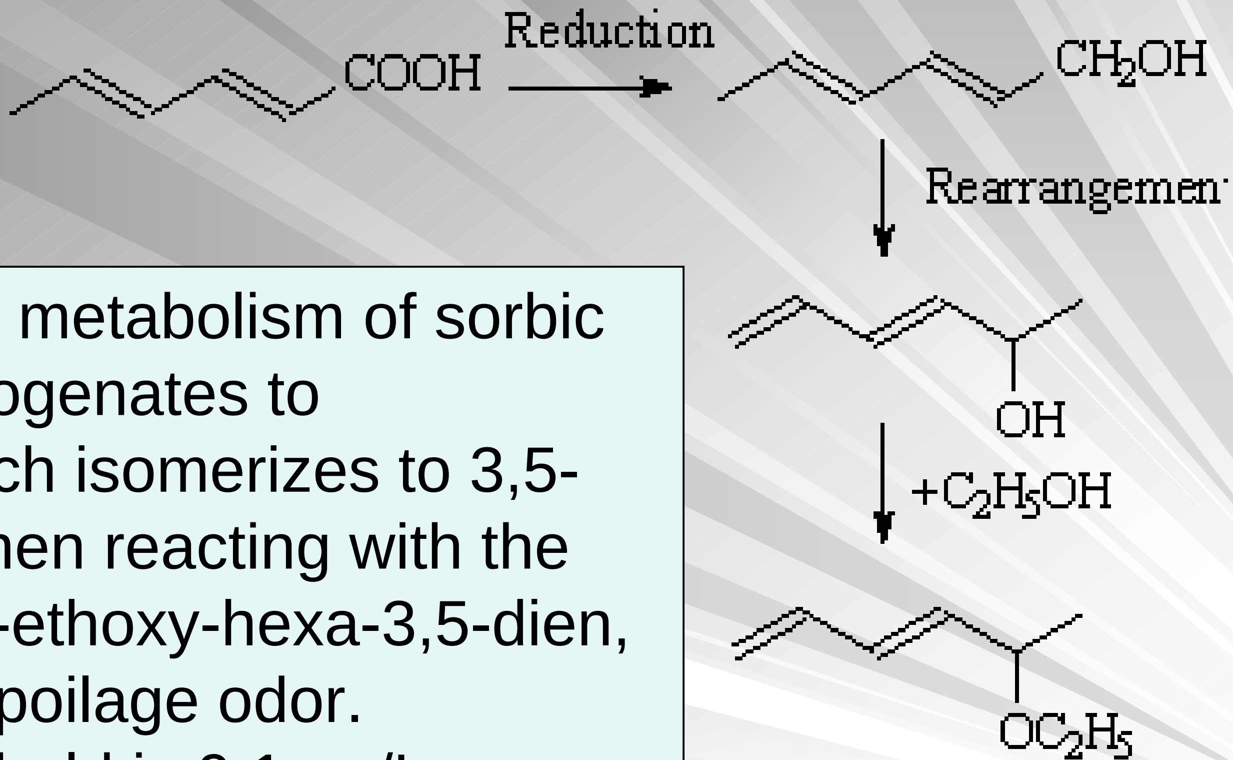
GERANIUM ODOR



- Derived from the metabolism of sorbic acid, which hydrogenates to SORBINOL, which isomerizes to 3,5-Hexadien-2-ol, then reacting with the ethanol part of 2-ethoxy-hexa-3,5-dien, resulting in the spoilage odor.
- Perception threshold is 0,1 µg/L.
- *O. oeni* has low a production.
- Not a problem in beverages without ethanol.

Diacetyl

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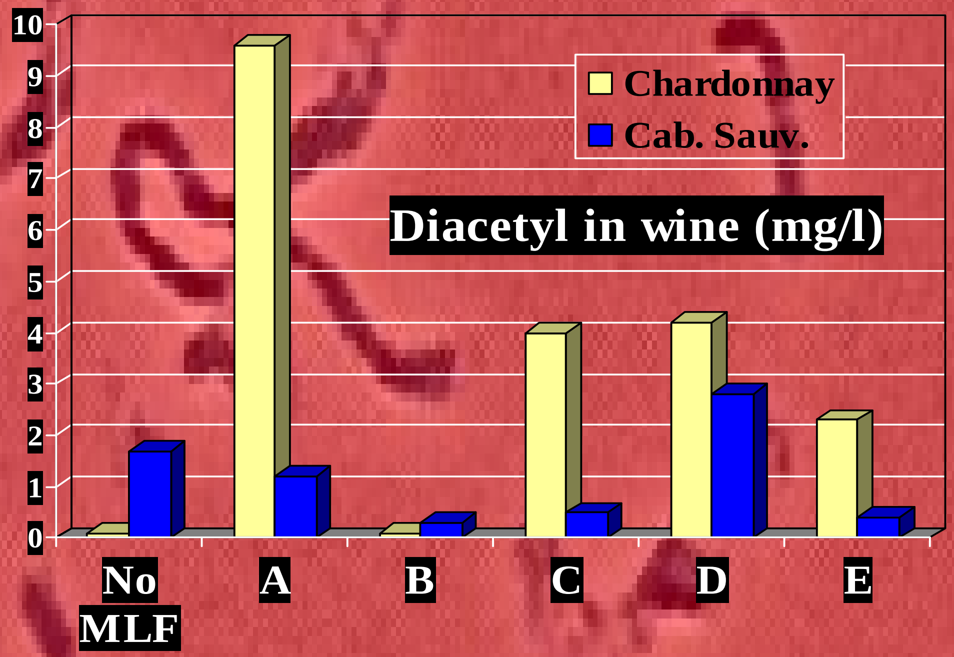
Diacetyl Impact

Bacteria Strain Diacetyl Production



- 5-14 mg/L
Butter
- 2- 4 mg/L
nutty
caramel
yeasty
honey
(threshold > reds)

The final concentration depends on the bacteria strain used for the MLF & on its citric acid metabolism

Metabolism of citrate by Oenococcus oeni and the transformation of diacetyl by yeasts

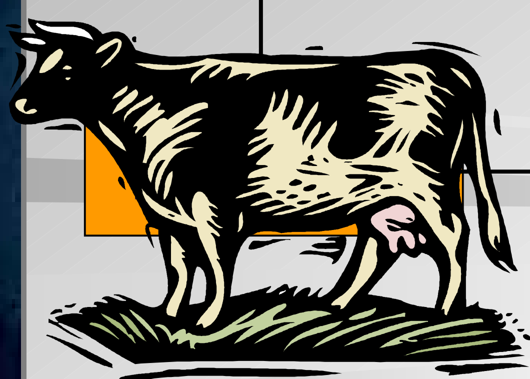
Citrate

Acetate

Oxalacetate

Pyruvate

The transformation of diacetyl to butandiol lowers the buttery aromas and transforms them into mouthfeel volume and fattness.



Acetoin

Biogenic Amines

MECHANISM OF BIOGENIC AMINE FORMATION

Biogenic Amines ← Decarboxylation ← Amino Acids ← Proteins

Decarboxylation of amino acids, Ex.: histidine decarboxylase for histamine

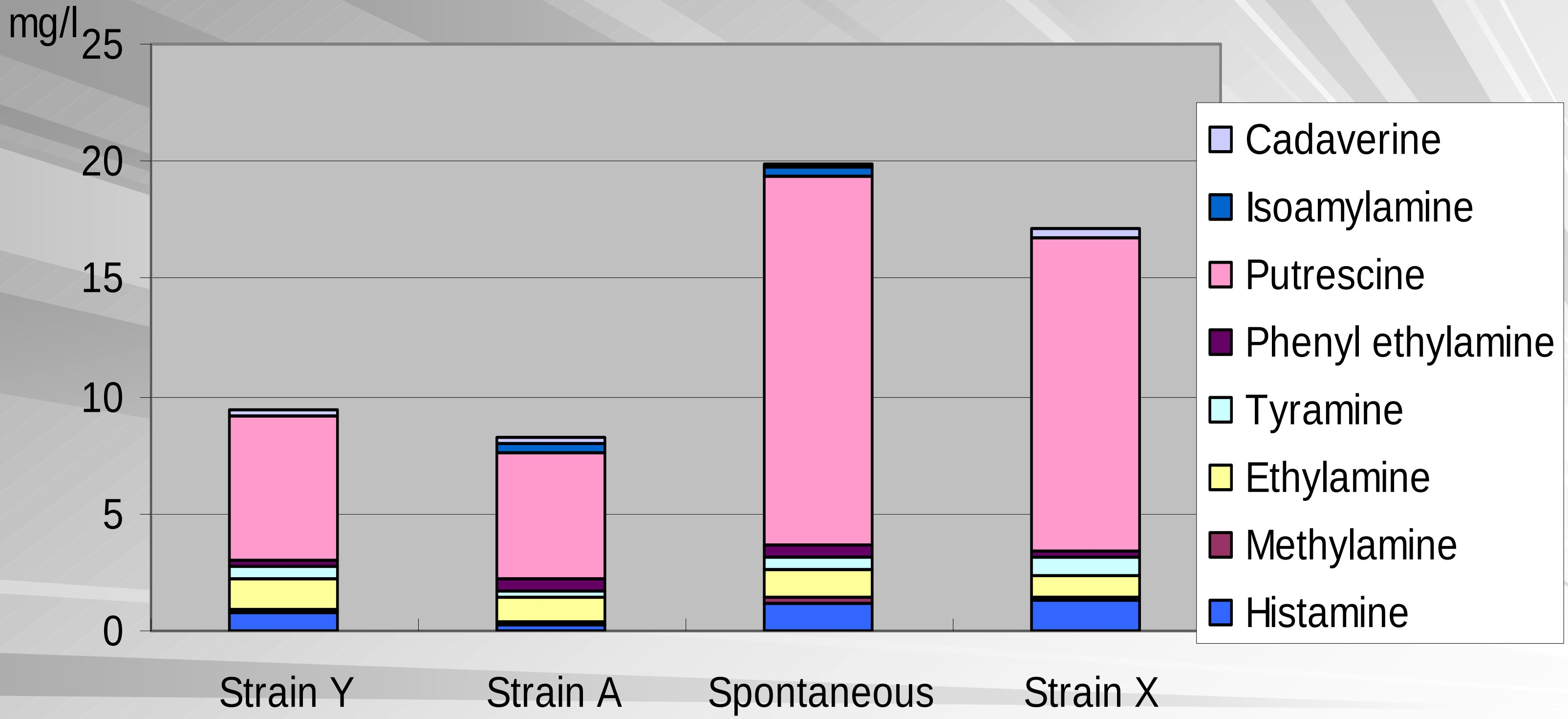
Biogenic amines are unhealthy (histamine) and also contribute negative sensory compounds (putrescine & cadaverine)

BIOGENIC AMINE FORMATION: examples

Amino acids – Biogenic Amines

Histidine	**Histamine**
Tyrosine	Tyramine
Lysine	**Cadaverine**
Arginine	**Putrescine**
Arginine	Espermine
Arginine	Epermidine
	Ethanolamine
	Phenylethylamine
	Isopentylamine

Lactic Acid Bacteria Strain Influence on Concentration of Biogenic Amines after MLF



Putrescine, a putrid "dirty sponge" aroma, is the highest contributor to Biogenic Amines in this trial.

Volatile Phenols

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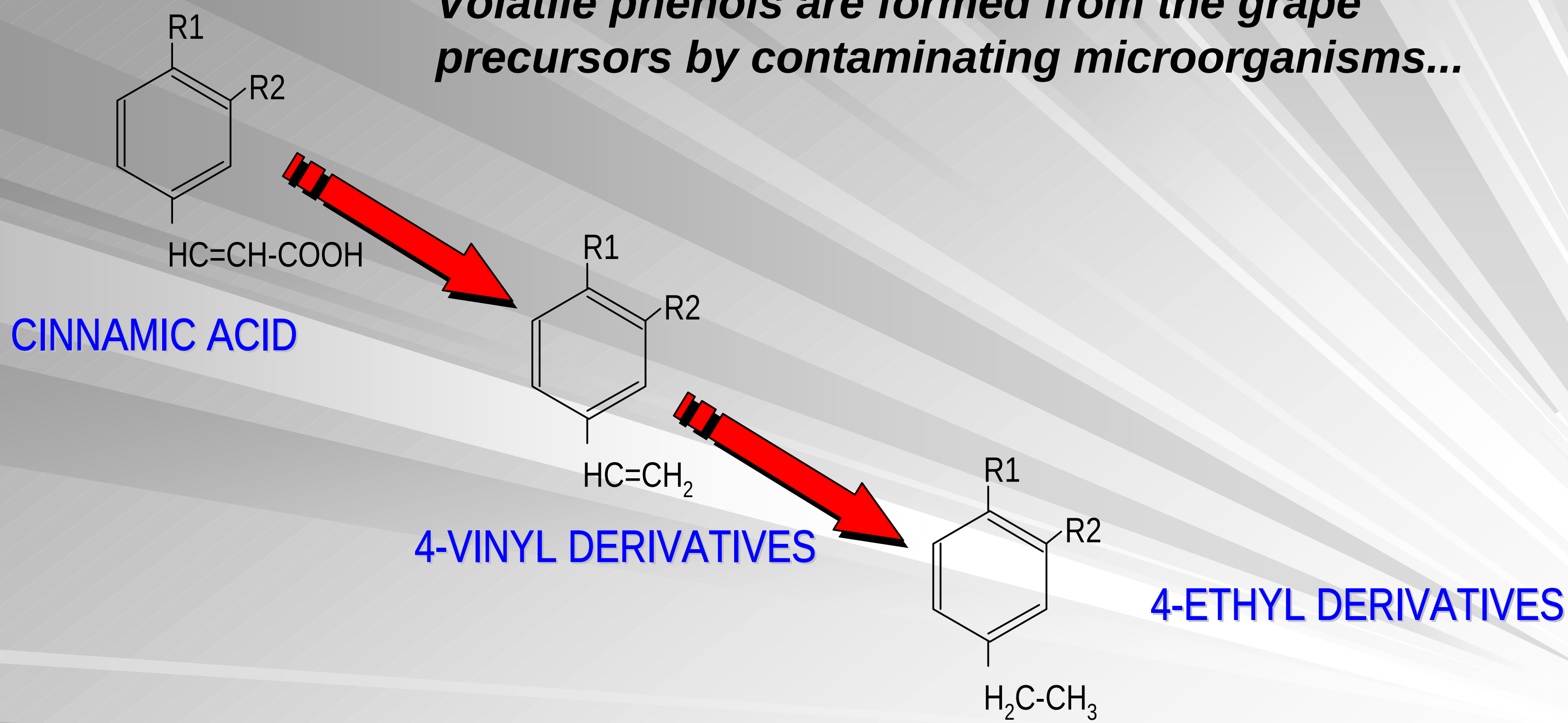
<u>*Lactobacillus*</u> ***can produce volatile phenols***

Volatile phenols are formed from the grape precursors by contaminating microorganisms...



Activity of *Lactobacillus plantarum*

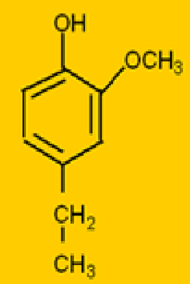
Brettanomyces Contamination...

The Problem

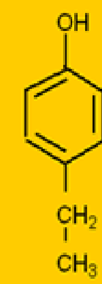
- **Contaminant yeast, responsible for the formation in wine of volatile phenols resulting in very negative aromas**

Ethylphenols 4-ethyl phenol
4-ethyl guaiacol
Vinylphenols 4-vinyl phenol
4-vinyl guaiacol

- **4- ethyl phenol results in the descriptors (“poorly cured leather”, “horse sweat”, “used socks”, “horse stables”).**



4-ethyl-guaiacol



4-ethyl-phenol

***Threshold perception*:**
Ethyl phenol: 600µg/L
Sum of ethyl phenols: 430 µg/L

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Mousy Off-Flavour

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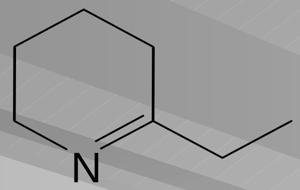
Causal compounds of Mousy off-flavour

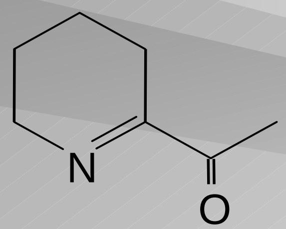
N-heterocyclic bases: 2-ethyltetrahydropyridine, 2-acetyltetrahydropyridine & 2-acetyl-1-pyrroline

2-ethyltetrahydropyridine (ETPY)



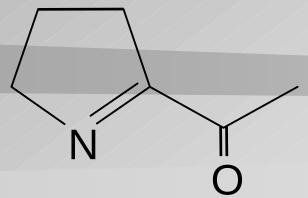
Taste threshold (wine): 150 µg/L (Craig & Heresztyn 1984)
Conc'n reported in wines exhibiting mousy off-flavour: 2.7-18.7 µg/L

2-acetyltetrahydropyridine (ACTPY)



Odour threshold (water): 1.6 µg/L (Teranishi et al. 1975)
Conc'n reported in wines exhibiting mousy off-flavour: 4.8-106 µg/L

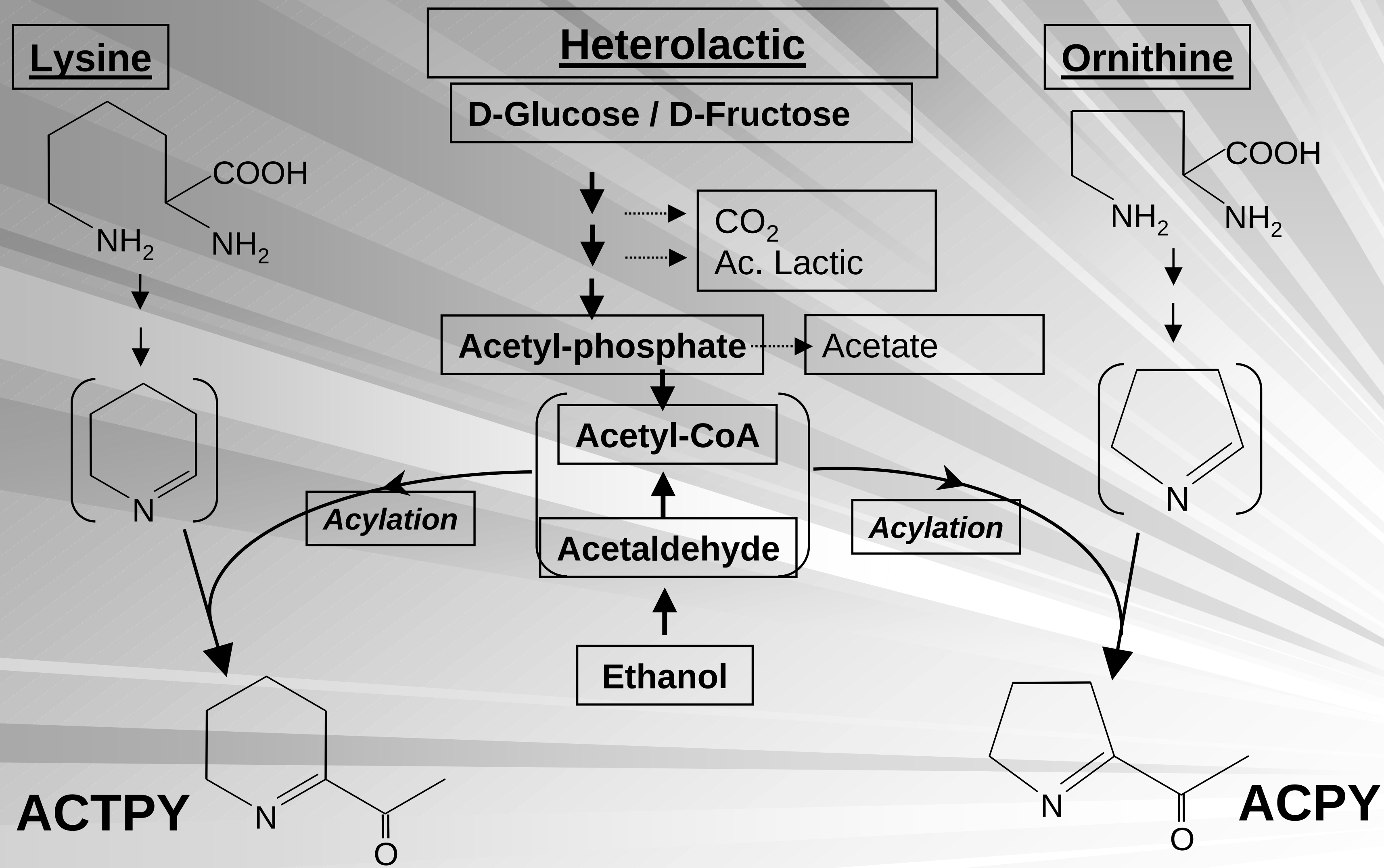
2-acetyl-1-pyrroline (ACPY)



Odour threshold (water): 0.1 µg/L (Buttery et al. 1983)
Conc'n reported in wines exhibiting mousy off-flavour: Tr-7.8 µg/L

Grbin, P.R.; Costello, P.J.; Herderich. M.; Markides, A.J.; Henschke, P.A.; Lee, T.H. (1996) Proceedings 9th Aust. Wine Industry Technical Conference, Adelaide, Australia, (Winetitles: Adelaide) pp.57-61.

Possible formation pathways of ACTPY & ACPY from *L. hilgardii DSM 20176*



Costello, P.J.; Henschke, P.A. (2002) J. Agric.Food Chem. 50: 7079-7087.

To avoid these “Masking” components…

- Limit the duration of lactic bacteria in the wine.
- Control the winery cleanliness to limit spoilage organisms (*Pediococcus, Lactobacillus*…)
- Use selected ML bacteria to control the MLF and avoid wine spoilage including biogenic amines.

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For more info please contact Sigrid@lallemand.com

Nitrogen requirements of yeasts

➡ Different demand depending on the strains fermentation temperature and pH

Jiranek *et al.*, 1991, Manginot *et al.*, 1998, Julien *et al.*, 2000

N2	N2	N2
71B / QA23 / DV10 / BC / EC8 / D47 / EC1118	EC7 / K1 / D254 / CAW / L2056 / R2 / RC212 / S6U	BDX / CSM / CY3079 / BM45 / K1 marque / L2226 / L2323 / VL1 / CEG

Nutrient requirements for *O.oeni*

Oenococcus oeni

(Leuconostoc oenos)

does not grow on

malic acid only

it needs complex nutrients

Improved nutrient formulations for Oenococcus oeni

Negative effects of the yeast on the bacteria (inhibition) could be caused by:

- Competition on nutrient level
- Production of inhibitory metabolites
  - SO_2
  - CO_2
  - Medium chain fatty acids
  - Antibacterial compounds

LALVIN MBR in difficult wine

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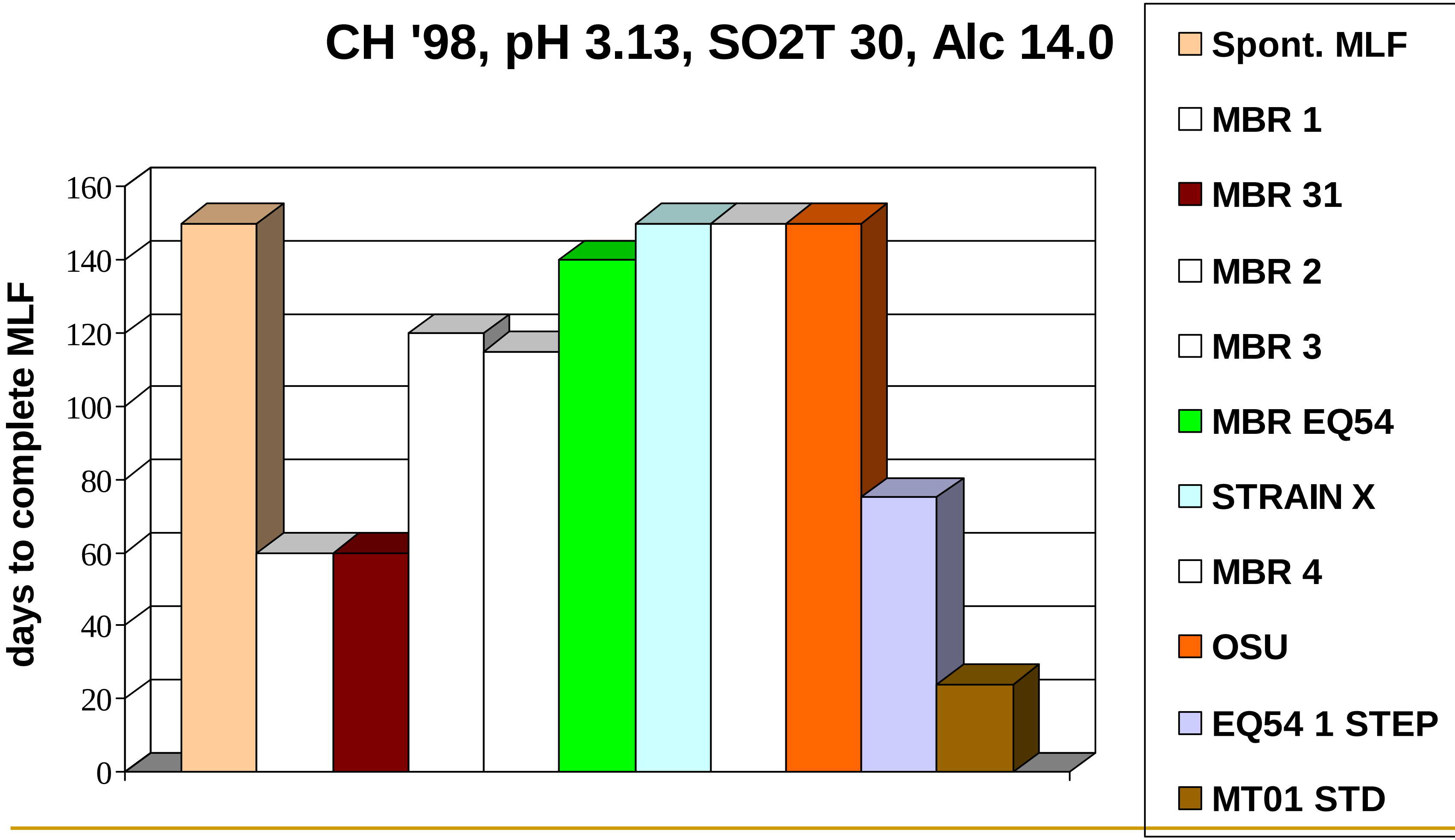
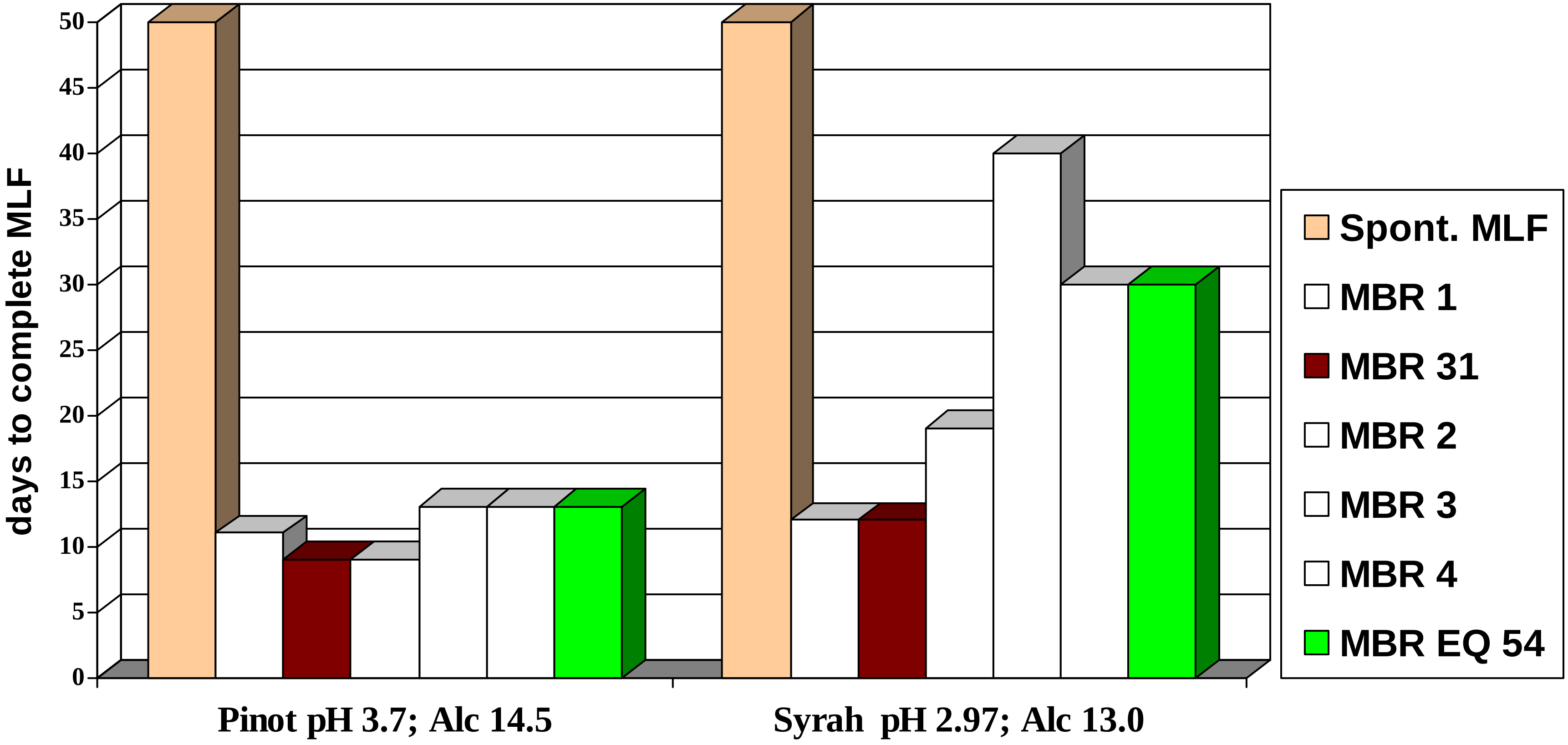


LALVIN MBR pH tolerance:

ITV Beaune, SOFRALAB '98



MBR®: Tolerance at low temperatures