Symposia

Quality And Safety Aspects In The Design Of Indigenous Wine Starters

Selecting LAB for use as starter cultures in winemaking

Patrick Lucas

Institute of Vine and Wine Sciences
University of Bordeaux
Grape

Alcoholic fermentation (AF)

Malolactic fermentation (MLF)

The LAB of MLF

Deacidification

Yeasts, Lactic acid bacteria

Malate

Lactate

+ Aromatic changes

Indigenous LAB

Selected LAB

> 10^6/ml

Spontaneous MLF

Controlled MLF

> 10^6/ml
Biogenic amines

Histidine

\[ \text{Histamine} \]

\[ \text{Histamine} \rightarrow \text{~30 mg/L} \]
\[ \text{No legal limit} \]
\[ \text{Commercial limits} \]

Indigenous LAB

Histamine-producing LAB (cells/ml)

\[ \text{Histamine} \rightarrow \text{(70% wines)} \]
\[ \text{Low risk} \ (30\% \ wines) \]
\[ \text{High risk} \ (70\% \ wines) \]

Ethyl carbamate (urethane)

\[ \text{Arginine} \rightarrow \text{H}_2\text{NCOCH}_3 \]

\[ \text{Usually <10 µg/L} \]
\[ \text{Legal limit (Canada) 30 µg/L} \]

264 wines. Spontaneous MLF

\[ \text{~30 \% indigenous LAB} \]

\[ \text{Usually <10 µg/L} \]
\[ \text{Legal limit (Canada) 30 µg/L} \]

Main origin: yeast urea
Indigenous LAB:

Unpredictable start and kinetics of MLF

other issues

Possible spoilages

Unpredictable aromatic impact

Mousy taint

Ropyness

+/− buttey

+/− fruity

+/− vegetal

Days

Malate (g/l)
Faster MLF
No spoilages
No histamine

(Better) controlled aromatic impact

Malate (g/l)

Days

Selected LAB

No MLF – Strain A – Strain B – Strain C

BEAUJOLAIS NOUVEAU
19TH NOVEMBER 2015
> 50 starters

<table>
<thead>
<tr>
<th>Company</th>
<th>Culture</th>
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<tr>
<td>AEB group</td>
<td>Biolact Acclimatée (3), Biolact Acclimatée BM (2), Biolact Acclimatée</td>
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<td>PB1025 (7), Biolact Acclimatée 4R (5), Biolact Fresh (4), Biolact One</td>
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<td>Fresh (6)</td>
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<td>CHR Hansen</td>
<td>Viniflora Oenos (1), Viniflora CH11 (1), Viniflora CH16 (1), Viniflora</td>
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<td>CH35 (1), Viniflora plantarum (Lactobacillus plantarum) (1), Lc-plantarum</td>
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<td>LpCHL2 (1)</td>
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<td>DSM food</td>
<td>Malolferm LG98 (1)</td>
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<td>specialties</td>
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<td>EverIntec</td>
<td>Extremo X 03 (2)</td>
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<td>Lallemand</td>
<td>Lacto350 Prege (1), Lacto350 Preue (1), Lacto350 SB3 Instant (1),</td>
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<td>Lacto350 B16 Standard (1), Microenos HP (Lb. hilgardii) (1)</td>
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<td>Téchard</td>
<td>Larvin 31 (1), Larvin EQS4 (1), Larvin VP41 (1), Uvaferm Alpha (1),</td>
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<td>Uvaferm Beta (1), Larvin PN4 (1), Larvin IB (Inobacter) (1), Larvin</td>
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<td>MT01 (1)</td>
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<td>Oliver Ogar</td>
<td>Malo Quick (1), Malo-start+ (1)</td>
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<td>SGBIOTECH</td>
<td>SNR (1), SNR (1), SNR (1), SNR (1), SNR (1), SNR (1), SNR (1)</td>
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<td>Tebaldi.it</td>
<td>ExperTi oeni (NS), ExperTi oeni Alcol (NS), ExperTi oeni pH (NS)</td>
</tr>
<tr>
<td>Vason</td>
<td>Amaro4 (1)</td>
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</tbody>
</table>

Increasing utilization

USA, France, Australia, Spain, Italy...

Beaujolais, Champagne, Bordeaux...

Selecting LAB starters for MLF

Natural LAB isolates

Essentially strains of *Oenococcus oeni*

- Good MLF kinetics
- Not producing biogenic amines, spoilages
- Tolerance to industrial production
- Resistance to « difficult wines »

**Spontaneous MLF**

- Acidity (pH +/- 3.5)
- Ethanol (+/- 13%)

**Lactobacillus plantarum**
**Lactobacillus malii**
**Lactobacillus kefiri**
**Lactobacillus lindneri**
**Lactobacillus brevis**
**Lactobacillus buchneri**
**Lactobacillus kunkeei**
**Lactococcus lactis**
**Enterococcus faecium**
**Enterococcus avium**
**Enterococcus durans**
**Enterococcus hermanniensis**
**Leuconostoc mesenteroides**
**Pediococcus parvulus**
**Pediococcus damnosus**

*Oenococcus oeni*
A growing demand for “wild” “terroir” strains

Wines
Wine estates
The origin as a selection criterion?
Wine regions
Diversity of *O. oeni*?
Specific strains?
Oenococcus oeni

1901 Bacteria of MLF
1967 First description

1980-90 First selected strains

Small cocci, pairs, chains Resembling Leuconostoc
Growth at low pH

Garvie, 1967 (J. Gen. Microbiol.)

2005 First genome
1.8 Mbp
Missing \textit{mutS}-\textit{mutL}

Mills et al., 2005 (FEMS Microbiol.)
Genus *Oenococcus*: alcohol-containing environments

**O. alcoholitolerans**: bioethanol

**O. kitaharae**: shochu

**O. oeni**: wine, cider ((fruits))

Adapted to live in wine (and cider)

16S tree adapted from Badotti et al. 2014 (A. Van Leeuw.)
A huge diversity of strains
**O. oeni** species population structure

**Multi Locus Sequence Typing**

- **Phylogenetic groups of strains**
  - A1
  - A2
  - B1
  - B2

- **513 strains**
  - Red or white wines, ciders
  - Worldwide

- **Bilhère et al, 2009 (AEM)**
- **Bridier et al, 2010 (AEM)**

- **Neighbour joining-tree**
  - 0.002 substitution/site

- **Cider, champagne, Chile, South Africa ...**
Diversity in regions

- 235 wines & ciders (ongoing MLF)
- Plating: 3200 LAB isolates
- Genotyping / MLVA: 514 O. oeni strains
- 100 X strains / region

70 – 87% “unique” strains in wine regions

Different diversity in regions

Are they genetically “region-specific”?

E Khoury et al, in preparation
Diversity in regions

514 strains: genetic grouping
50 genome sequences

+ Sequenom MassArray iPLEX platform

⇒ SNP-based genotyping
⇒ Localisation in phylogroups

⇒ In most regions, *O. oeni* strains are not genetically-adapted
⇒ Genetic adaptation to cider or wine? White or red wines?

E Khoury et al, in preparation
Comparative genomics

50 *O. oeni* genomes
France, Italy, USA, Australia ...
Cider, red wine, champagne ...

3 *Leuconostoc*
4 *O. kitaharae*

Adaptation to products?

Genetic adaptation to cider, wine and certain types of wines

*O. oeni* genotypes

*BMC Genomics*
Genetic adaptation to red or white wines

Campbell-Sills et al., in preparation

Comparative genomics

Whole genome alignment (ANI)

Heat map of gene repertoires

+ 15 O. oeni from the red & white wine groups

- 2 groups confirmed

- 2 sister groups that evolved from a common ancestor

- Specific gene contents (sugars utilization, stress resistance)

- Genetic adaptation to red or white wines
What about the wine they produce?

Metabolomics
4 strains from each group
MLF of a Chardonnay wine
GC-MS / volatile compounds

PARAFAC model
Component 2: 12.18 % expl. var.
Component 4: 7.77 % expl. var.

- Similar wines with strains of a same group
- Different wines with strains of different groups

Control

- Congruence loadings segment mode
Conclusion: the origin of strains must be considered

- High genetic diversity in regions
- Strains mobility between regions
- But the diversity in each region is “unique”: there are regional microbiological terroirs
- At least in some cases, there are specific strains
- Similar situation (diversity, uniqueness)
- Several strains remain from year to year
- Product-specific strains (pH, acidity, phenolics, ...)
Acknowledgements

Organizing committee

Symposium:
Dr. Aspasia Nisiotou
ELGO “DEMETER”, Institute of Technology of Agricultural Products

Students, lab members

Dr. Mariette El Khoury
Ecology

Cécile Miot-Sertier
Olivier Claisse
Lucie Dutilh

Dr. Hugo Campbell-Sills
Bioinformatics, metabolomics

Project partners

Project WILDWINE 2012-2015
FP7 SME-AG

Dr. Chrysoula Tassou
ELGO “DEMETER”, Institute of Technology of Agricultural Products

Project LevainsBio 2012-2015