Use of wild yeast and bacteria in wine fermentation

Brewing Short Course &
REG Meeting
Diversity of yeast and bacteria

- 11 to 20 different yeasts, every genus with sometimes more than 5 species
- 5 to 7 relevant bacteria including several species
- Survival factors in the vineyard are completely different from the cellar

**Yeast Genus**
- *Hanseniaspora*
- *Saccharomyces*
- *Pichia*
- *Candida*
- *Saccharomycodes*
- *Dekkera*
- *Debaromyces*
- *Metschnikowia*

**Bacteria Genus**
- *Oenococcus*
- *Pediococcus*
- *Leuconostoc*
- *Lactobacillus*
- *Acetobacter*

... and many more
Survival factors of microorganisms

In the vineyard
- High oxygen
- No alcohol
- Low sugar levels
- High temperatures

In the cellar
- Very little oxygen
- Increasing ethanol level
- High sugar levels
- Low temperatures
Spontaneous fermentation without SO$_2$

Candida stellata

Hanseniaspora uvarum

„wild yeast“

Metschnikowia pulcherrima
Typical Flavor caused by wild yeast strains

negative

• band aid
• oxidized (acetaldehyde)
• medically
• volatile acidity and ethylacetate
• hydrogensulfide
• „Mousy-taint“ impressions
• Lower extract and lactic flavors due to flor yeast
• sweaty, fleshly
• Petroleum and kerosene aromas

positive

• Creamy character
• Complex acidity composure
• Intense mouth-feel
• Exotic fruit
• diverse fruity esters
• Sulfur containing aroma compounds

fruity, highly complex wines due to the contribution of different micro-organisms
Does spontaneous fermentation lead to more interesting wines?

Parallel spontaneous and inoculated fermentation of Riesling in wineries (yeast: R-HST, Lallemand)
Aroma profile with fast fermentation

- Spontaneous fermentation considered more fruit and body
- Positive compared to control

→ Fermentation time ~ 30 days
Aroma profile with slow fermentation

- Pure culture yeast judged more fruity with similar body and bitterness
- Reduced effect of wild yeast flora

→ Fermentation time ~ 60 days
Cell count and sugar degradation

- The higher the cell count, the faster the fermentation
- The higher the temperature, the faster the proliferation
- The slower the proliferation, the lower the final cell count
Danger zone malolactic fermentation

How great is the risk of unwanted bacterial activity in spontaneous fermentations?
Comparison of 2 wines from the same must

- Spontaneous malolactic fermentation can lead to buttery and Sauerkraut-like characters

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Mineralic
7

Bitterness
6

Lemon
5

Mouthfeel
4

Apple
3

Body
2

Elder flower
1

Acidity
0

Peach

Passionfruit

Buttery/Cheesy

Green bean

Green grass

Smokey

Honeymelon

Small Scale

Winery
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Effects of spontaneous MLF

- Increase in smokey, green, and buttery attributes
- Increase in bitterness
- Decrease in perceived fruity aromas
MLF during spontaneous fermentation

- Speed of bacterial growth is depending on temperature
- Only delay in MLF, no inhibition by lower temperatures
Wild flora towards the end of fermentation

*Lactobacillus brevis*
*Lactobacillus hilgardii*

Decreasing yeast activity
Risk of unwanted MLF
Conclusion I

- With fast metabolic rates spontaneous fermentation can increase the complexity of wines.
- With slow metabolic rates (>60 days) spontaneous fermentations tend to mask the fruity character of wine.
- Cooler temperatures support growth of non-\(\text{Saccharomyces}\) yeasts and increase the risk of spoilage or stuck fermentation.
- Higher temperatures in the beginning increase the chance of early fermentation start and high \(\text{Saccharomyces}\) cell counts.
- The risk of unwanted MLF should be controlled with lysozyme; addition of \(\text{SO}_2\) would inhibit non-\(\text{Saccharomyces}\) flora and change the composition.
- Strategies to conduct spontaneous fermentation must be adapted to the desired style of wine!
Does spontaneous fermentation enhance or mask the Terroir character of wine?

- Wild yeast strains may contribute to complex aroma impressions, but also to off-flavor formation

- **Hypothesis:** Terroir is influencing the composition of the wild yeast flora
Why connecting Terroir and Yeast?

Terroir character

- Soil
- Cover crop
- Sunshine
- Slope
- Wind
- Rain/Water
Why connecting Terroir and Yeast?

Almost the same factors have an impact on the yeast flora in the vineyard!

- Sunshine
- Soil
- Cover crop
- Wind
- Rain/Water
Experimental setup

3 different wines from one vineyard
- Spontaneous fermentation in the winery incl. Cellar-yeast (referred to as winery)
- Spontaneous fermentation small scale only with yeast from the vineyard (small scale)
- Inoculation of pasteurized must (inoculation)
Sensory evaluation of all experiments

Except for a few wines, grouping is possible due to sensory properties.
Small scale spontaneous fermentation

- Only little differences in fruity characters

Differences due to terroir and site specific yeasts (?)
Spontaneous fermentation in the wineries

- Differences between fruity characters
- No differences in negative parameters

Differences by including the „cellar yeast“
Why do winery wines produce other profiles than „sterile“ small scale fermentation?

- Similar sensory results in four different vintages

- **Hypothesis**: yeast flora from the cellar dominates sensory profile.

- **Proof**: Identification and quantification of the yeast species involved
Identification of species with DGGE

- Differentiation on species level
- No absolute quantification; educated guess

Harvested yeast

Extract and wash DNA

DNA Mix of different species

Polymerase Chain Reaction

Compare with Single cultures

Quantify by evaluating fluorescence intensity

DGGE procedure according to Mills et al., 2000
Yeast dynamics in spontaneous fermentation

- At 18°C fermentation starts rapidly
- *Saccharomyces* takes over after 6 days
- Part of *Hanseniaspora* remains active for a longer period
- Delayed onset of fermentation doubles growth time for „wild yeast“!
Spontaneous flora without SO₂ addition

- Composition of yeast population changes with increasing ethanol concentration
- Above 4 %vol. ethanol only \textit{Saccharomyces} active

Modified from Großmann et al. 2005
Conclusions II

- Spontaneous fermentation show higher batch-to-batch variation
- Cooler temperatures favor growth of wild yeast and yields a different aroma profile
- Composition of the wild yeast flora varies only slightly between sites
- Spontaneous fermentations in the wineries had more Saccharomyces yeasts than those fermented under sterile conditions (cellar yeast flora)
- The individual cellar yeast flora seems to dominate the final sensory properties of wines from spontaneous fermentations and not the flora of individual vineyard sites
Impact of yeast composition on the flavor profile of wine?

• **Hypothesis:** Variation among volatiles in finished wines is related to yeast composition during fermentation.

• Use of analytical fingerprints to display possible differences
Analytical „Fingerprints“

- Comprehensive two dimensional gas chromatography yields high separation of complex mixture of volatiles
- Fingerprinting method to display differences between wines
Volatile explaining the most variation

- Selection of 23 compounds out of 283 based on explained variability

Mainly:
- Alcohols
- Esters
- Hydrocarbons

Variables (Axes F1 and F2: 79.09 %)
Variation among wines due to volatiles

- Dried active yeast and inoculations together
  - Influence of wild yeast strains less important
  - Dominance of Saccharomyces
- "pure" spontaneous fermentation batches on one side
- More variation among winery wines than among "sterile" batches
  - Impact of cellar flora increases differences between wines
How to make it work in your cellar?

What to recommend to the winemakers?
Ways to conduct spontaneous fermentation

„Real“ spontaneous fermentation
random population of microorganisms from vineyard and cellar

„Russian Roulette“
Ways to conduct spontaneous fermentation

Conducted

Spontaneous fermentation I

Yeast from successful spontaneous fermentation used to inoculate other batches

"Real" spontaneous fermentation

Random population of microorganisms from vineyard and cellar
Ways to conduct spontaneous fermentation

„Real“ spontaneous fermentation
random population of microorganisms from vineyard and cellar

Conducted spontaneous fermentation I
Yeast from successful spontaneous fermentation used to inoculate other batches

Conducted spontaneous fermentation I
(Pied de Cuve)
Early harvesting of healthy grapes to establish a starter culture for further inoculation
Ways to conduct spontaneous fermentation

„Real“ spontaneous fermentation
random population of microorganisms from vineyard and cellar

Conducted spontaneous fermentation I
Yeast from successful spontaneous fermentation used to inoculate other batches

Conducted spontaneous fermentation I (Pied de Cuve)
Early harvesting of healthy grapes to establish a starter culture for further inoculation

„optimized“ spontaneous fermentation
spontaneous start followed by inoculation with dried active yeast

„Diversity due to wild yeast, safety by using Saccharomyces“
Be on the wild or safe side of fermentation

"Real” spontaneous fermentation
random population of microorganisms from vineyard

"optimized” spontaneous fermentation
spontaneous start followed by inoculation with dried active yeast

No significant difference compared to dried active yeast fermentation

High Risk of stuck fermentation Low

• "optimized“ spontaneous fermentation including established cellar yeast recommendable

• => influence of cellar yeast seems to be much more important for the final flavor composition than wild yeast from the vineyards
Yeast and bacteria co-inoculation

Focusing on diacetyl
Diacetyl in wine

- Dicarbonyl compound with distinct buttery flavor
- Sensory threshold 0.2 mg/L – 2.8 mg/L depending on wine matrix
- Higher concentrations are considered as off-flavors especially in white wines
- Formation and degradation by yeast and bacteria possible → final concentration as a result of complex metabolic activity
- Literature relates diacetyl formation to citrate degradation during MLF
  → Use of bacteria strains that lack the responsible enzyme (citrate lyase negative, CL
  \( \text{neg} \))
CiNe (Eaton Begerow, Germany) leaves citrate untouched

VP 41 (Lallemand, Canada) is supposed to metabolize citrate without diacetyl formation

... but some of the wines contained diacetyl well above the sensory threshold!
Citric acid degradation

Bacteria

Uptake towards the end of MLF

Constant release throughout growth

Release of reduced diacetyl strain depending

Yeast

From yeast sugar metabolism

Release during amino acid synthesis (valine)

Uptake and reduction to 2,3-butanediol

adapted from Quintans et al., 2008
Pinot blanc 2011 and 2012

- Fermented with yeast CY 3079 (Lallemand)
- Divided in simultaneous and consecutive MLF
- Trials run in duplicates

Control no MLF
- CiNe (Eaton’s Begerow)
- Citrate lyase negative

Medium production
- CLNX (FermControl)
- VP 41 (Lallemand)

Strong production
- ALPHA (Lallemand)
- BETA (Lallemand)
Influence of cell concentration

- no MLF with less than $10^6$ CFU/mL
- some commercial products contain less viable cells
- delayed MLF
- risk of undesired flavor development
Accumulation of diacetyl

- No reduction of the diacetyl formed by bacteria $\rightarrow$ accumulation
- Reason: low pH prior to fermentation (2.9) delays MLF and citrate degradation $\rightarrow$ higher final level of diacetyl
Diacetyl concentration during vinification

- First increase induced by both yeast and bacteria
- Second increase mainly caused by *O. oeni*
- Correlation between biomass production and diacetyl accumulation
Gene expression analysis (qRT-PCR)

- Citrate transporter (*maeP*)
- Citrate lyase (*citE*)
- Lactate dehydrogenase (*ldh*) → House-keeping gene
- Acetolactate synthase (*alsS*)
- Acetolactate decarboxylase (*alsD*)
- Acetoin reductase (*butA2*)

adapted from Quintans et al., 2008
Gene expression analysis (qRT-PCR)

- first \(alsS\) over-expression induced by sugar metabolism
- second \(alsS\) over-expression induced by citrate degradation
The central role of pyruvate

Pyruvate addition
The central role of pyruvate

→ Degradation von citrate also leads to an increase in pyruvate
**Diacetyl degradation by yeast**

- Diacetyl addition does not affect growth or MLF rate, even in high concentrations.
- The dried active yeast strain CY 3079 (Lallemand) is able to reduce 50 mg/L diacetyl in the first two days of fermentation.
Independent of the start concentration 2 mg/L diacetyl remain due to bound diacetyl (to sulfur dioxide, amino acids and others)
What does that mean for the winemaker?

- Only a rapid MLF can inhibit diacetyl accumulation
- At least $10^6$ CFU/mL are required to start MLF $\Rightarrow$ cell count after inoculation if commercial preparation is unknown
- Initial medium must be suitable for MLF (pH at least 3.1-3.3) $\Rightarrow$ higher pH might favor growth of other bacteria (e.g. *Pediococcus damnosus*) and therefore diacetyl formation $\Rightarrow$ lower pH delays MLF and therefore favors diacetyl formation
- Once diacetyl is formed curative measures like fresh yeast fining may be applied
Dried active yeast (CY 3079) is able to reduce 50 mg/L diacetyl in two days. Independent from the starting level, residual diacetyl of 2 mg/L always remains; reason possibly bound diacetyl.

Correlation between biomass increase and diacetyl accumulation observed (more bacteria produce more diacetyl)

Over-expression of alsS gene due to high concentrations of pyruvate as well as citrate

Formation of diacetyl by citrate degradation only can be excluded

Dried active yeast (CY 3079) is able to reduce 50 mg/L diacetyl in two days.

Independent from the starting level, residual diacetyl of 2 mg/L always remains; reason possibly bound diacetyl.

→ Diacetyl accumulation shows two characteristic peaks: first increase caused by yeast and bacteria, second increase due to bacteria only.

Summary and conclusion