Influence of different management practices on the fungal and bacterial biota of carpospheres of ripening grape clusters

Elizabeth Kecskeméti, Beate Berkelmann-Löhnertz and Annette Reineke
Geisenheim University
Institute of Phytomedicine
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Recent increase of disease pressure of grape bunch rot (*Botrytis cinerea* et al.) due to climate change!
Interactions between microorganisms being present on grape berries can influence the extent of *Botrytis* disease epidemics and may have effects on bunch rot control strategies.
Microbial biodiversity on grape berry skins - Questions

- Can we detect a **change in microbial communities** on grape berry skins during the ripening period?

- Do **different management systems** have an influence on the abundance of microorganisms on grape berry skins?

- Are any **microbial antagonists** present on grape berry skins and is their presence influenced by different management systems?
Sampling site: cv. Riesling, 2010 and 2011

Integrated viticulture

Synthetic fungicides
Sulphur

Copper and Sulphur
Plant resistance inducers

Biodynamic preparations like cow manure

Bioorganic & Biodynamic viticulture
Sampling site: cv. Riesling, 2010 and 2011

integrierte Bewirtschaftung

grapevine plants sampled
Sampling of berries (2010 and 2011)

BBCH 81 – beginning of ripening
BBCH 85 – softening of berries
BBCH 89 – berries ripe for harvest

Date 1 BBCH 81
Date 2 BBCH 85
Date 3 BBCH 89

healthy berries
increase of bunch rot
Extraction of DNA and PCR

DNA Extraction

Berries + H₂O-Tween
down
3 min Sonication
down
30 min Shaking
down
Centrifugation
8000 rpm, 30 min, 22 °C
down
Store pellet at -20 °C
down
DNA Isolation
(PowerSoil® DNA Isolation Kit)
**Extraction of DNA and PCR**

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**DNA Pooling**
- DNA aliquots of 2 samples per site and date
- = 72 pooled samples
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**Amplification**
- 72 samples using fungal ITS primer
- 72 samples using bacterial 16S rDNA primer

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454-Sequencing

- LGC Genomics GmbH
  Bacteria: 46,100 reads
  Fungi: 98,900 reads
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**Data analysis**
Fungal diversity during grape ripening 2010

Increase of abundance of members of Sclerotiniaceae (*Botrytis* et al.) during grape ripening period.
Analysis of similarities (ANOSIM) between samples: Fungal communities present at the same BBCH stage on grape berries are similar between different management systems.
Analysis of similarities (ANOSIM) between samples: Significant difference in the composition of fungal communities between samples from different management practices at **BBCH 89**, higher diversity in samples from integrated vineyard plots.
Four most abundant fungal species

**BBCH 81**: Significant difference in abundance of *Alternaria alternata* between management practices

**BBCH 89**: Significant difference in abundance of four most common fungal species between years
Bacterial diversity during grape ripening 2011

Analysis of similarities (ANOSIM) between samples: Significant differences in the composition of bacterial communities on grapes between samples from different management practices at BBCH 81 and 85.
Analysis of similarities (ANOSIM) between samples: Significant differences in the composition of bacterial communities on grapes between samples from different management practices at BBCH 81 and 85.
Five most abundant bacterial species integrated bioorganic biodynamic

**BBCH 81**: Significant difference in abundance of *Sphingomonas* and *Pseudomonas* between management practices

**BBCH 85**: Significant difference in abundance of *Pseudomonas* spp. between management practices
Microbial biodiversity on grape berry skins – Questions and first answers

✓ Can we detect a change in microbial communities on grape berry skins during the ripening period? Yes, abundance of members of the Sclerotiniaceae (Botrytis cinerea) increases, extent of fungal biodiversity decreases.

✓ Do different management systems have an influence on the abundance of microorganisms on grape berry skins? Yes, but dependent on species and year.

➢ Are any microbial antagonists present on grape berry skins and is their presence influenced by different management systems?
Presence of putative fungal antagonists

- Aureobasidium pullulans
- Metschnikowia pulcherrima
- Pichia membranifaciens
- Sporidiobolus pararoseus
- Cordicipitaceae u. a. Beauveria bassiana (entomopathogenic fungus)

Presence of antagonists dependent on year and management system, high abundance of *A. pullulans* on grape berries from integrated vineyard plots.
Putative fungal antagonists - qPCR

Fungal reference genes

Integrated bioorganic biodynamic

High variation in abundance of *A. pullulans* and *S. pararoseus* between samples from single grapevine plants
Relative quantities of *S. pararoseus* significantly higher on grape berry samples obtained from integrated compared to those from biodynamic vineyard plots ($p = 0.0028$).
Presence of putative bacterial antagonists and extent of diversity different depending on management system and year; in general high abundance of *Pseudomonas* spp.
Microbial biodiversity on grape berry skins – Questions and answers

✔ Can we detect a change in microbial communities on grape berry skins during the ripening period? Yes, abundance of members of the Sclerotiniaceae (Botrytis cinerea) increases, extent of fungal diversity decreases.

✔ Do different management systems have an influence on abundance of microorganisms on grape berry skins? Yes, but dependent on species and year.

✔ Are any microbial antagonists present on grape berry skins and is their presence influenced by different management systems? Yes, but dependent on species and year; significantly higher abundance of Sporiodobulus pararoseus on grape berry samples from integrated vineyards (qPCR).
Overall: 454 Pyrosequencing is an efficient and high-throughput method to analyse microbial communities on grapes, might have a high potential to answer future questions on the influence of plant protection strategies on (microbial) biodiversity in vineyards.
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... and you for your attention!