Management through the Microbiome: How Manipulating Grape Endophytes Can Affect Berry Development

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Where we’re going

- Current Research in my Lab
- Background on the Microbiome
- Modifying the Microbiome
- Results
- Future Avenues of Research
- Questions
Current Research in my Lab
• Sour rot
  • Fine tuning spray timings
  • Implicating larvae in SR progression

• Grapevine Trunk Disease
  • Prevalence in MO
  • Variety variation

• Ripe rot
  • Prevalence
  • Management strategies

• Phomopsis
  • Prevalence
  • Management strategies

• Trialing 5 MN varieties
  • Good luck with Viognier and Petit Manseng
Overwintering stage of Phomopsis

Bleaching
Black spots

Management:

Dormant spray: Sulforix or lime sulfur

1” – 3” shoot growth: Mancozeb or Captan
Background on the Microbiome
Yeast and Bacteria are prevalent in healthy grapes
The grape berry is a factory for various biochemical compounds.

Flesh contains the most juice.

Seed:flesh ratio depends on variety and climate.

Number of seeds depends on variety and climate.
Flavonoids are produced in seeds and skins

• Important for the color and taste of wine
  • Tannins and anthocyanins are the major flavonoids
Two stages:
- Berry formation
- Berry Ripening

Xylem supplies berry early in season (water, minerals, nutrients)

Phloem supplies berries after Veraison (photosynthates/sugars)
Berry formation
First stage of growth: Bloom + 60 days

• Berry expands
• Tartaric and malic acids accumulate
  • Provide the acidity in wine
• Hydrocinnamic acids accumulate
  • Precursors to volatile phenols
    • Each volatile phenol has a distinct aroma (clove, sweat, etc...)
• Tannins begin to accumulate
  • In seed and skin, not flesh
  • Contribute to color stability
Berry Ripening
Second stage of growth: softening & color change

- Berry doubles in size from Veraison to harvest
- Malic acid, tannins, certain volatiles (methoxypyrazines) decline (and not just by dilution)
- Huge increase in glucose and fructose
  - Sucrose produced by photosynthesis
  - Transported into the berries
  - Hydrolized into glucose & fructose
- Secondary metabolite production
  - Anthocyanins (skin)
  - Volatiles (flesh and skin)
Even though everyone claims that wine is made in the vineyard...

- Overcropping/undercropping
- Pyrazines are thought to decline with sunlight exposure (leaf pulling)
- Hanging fruit longer (more sugar)
- Earlier harvest (more acid)
...the real toolbox has generally been in the winery

- Yeast impart many characteristics (aroma, mouthfeel)
- Enzymes
- Adding tannin (tannin products derived from grapes and oak are common)
- Adding acid
- Adding sugar
- Maceration (pump-overs, punchdowns, submerged cap, pulsed air)
- Oak chips
- Fining (bentonite, PVPP, gelatin, egg whites, casein)
- Aging
But terroir is important, right? 
...so what is it?
Terroir

Three components:
- Soil
- Climate
- Cultivar

- The environment relates to sensory attributes in wine, but how?
- Terroir is hard to study!
- Microbes are one way of studying terroir
  - High-throughput sequencing
What about microbes? They aren’t usually mentioned when discussing the grape berry biochemistry. But they must be playing a role in grape growing.
Most research has been on must and grape surface

Challenges isolating DNA from the grape surface

Thick, waxy cuticle and limited amount of DNA
Development of DNA extraction technique
Microbes vary by region

Fig. 1. Grape must bacterial communities demonstrate distinct regional patterns. (A) Weighted UniFrac distance dendrogram comparing bacterial communities of Chardonnay musts from across California. Branches are colored by the growing regions they represent, while branches encompassing the same growing region are highlighted in the same color. (B) PC analysis showing the relative contributions of each principal component (PC) to the variation in the bacterial communities. (C) Scatter plot showing the distribution of bacterial communities across different growing regions. (D) Phylogenetic tree showing the relationships between different bacterial species.
Microbes vary by cultivar
Healthy grapes have an abundance of microbes in their pulp

Can we manipulate the grape microbiome?

What’s the best timing?

Will manipulating the microbiome change the berries in any way?
Manipulation study

- **2018**: Research vineyard: 3 reps of vines each of *Vitis* interspecific hybrid cvs. Chardonel and Norton sprayed at Bloom with 4 different single-species active yeast or water (control)
- Berry weight, rachis length, cluster compactness all done at Veraison and Harvest
2018 Total berry weight (g)

Yeast 1  Yeast 2  Yeast 3  Yeast 4  Control

Chardonel  Norton

Student t’s, p = 0.05
2018 Number of berries

Yeast 1
Yeast 2
Yeast 3
Yeast 4
Control

Chardonel
Norton

Student t's, p = 0.05
2018 Rachis weight (g)

Student t’s, p = 0.05
2018 Cluster compactness (berries/cm)

Yeast 1
Yeast 2
Yeast 3
Yeast 4
Control

Chardonel
Norton

Student t’s, p = 0.05
• Yeasts 1 and 3 made an impact on Norton
  • Larger and more berries

• No *significant* impacts on Chardonnel, but impacts nonetheless
  • Yeast 2 and 4 showed larger and more berries
Relative Distribution of Orders of the 20 most Abundant Taxa

Similarities within treatments
Variation between treatments
Treated vines looks different than the Control
2019: Garnering grower support

- Missouri Department of Agriculture, Specialty Crop Block Grant: “Determining the impact of the grape endophytic microbiome on grape physiology.” M. E. Hall (PI). Requested years 1 and 2 (10/01/19-09/30/21): $38,010. Awarded.
2019: Commercial collaboration

- **2019:** 2 Commercial Vineyards: 5 reps of single vines of Vitis interspecific hybrid cv. Vignoles sprayed at Bloom with 4 different single-species active yeast or water (control)
- 10 clusters harvested from each vine for all 25 vines
- For 15 vines (3 reps), entire vine was harvested
- Cluster weight, berry weight, rachis length, cluster compactness all done at Veraison and Harvest
- Wines made from each treatment using inoculation of commercial yeast
Average Total Berry Weight affected

2019 Avg. Total Berry Weight (g)

Yeast 1  Yeast 2  Yeast 3  Yeast 4  Control (H20)

Vineyard 1  Vineyard 2

Student t’s, p = 0.05
Rachis length affected

2019 Rachis length (cm)

Yeast 1  Yeast 2  Yeast 3  Yeast 4  Control (H2O)

Vineyard 1  Vineyard 2

Student t’s, p = 0.05
• Yeasts 2 and 4 significantly affected the Vignoles
  • Larger berries, longer clusters, and more berries
• Yeast 4 reduced cluster compactness in Vineyard 2
• Consistent with the Chardonel results
Fermentation time affected

![Bar chart showing length of fermentation in weeks for different yeast strains and control (H2O).]

No error bars because all samples finished on the same days in each treatment.
Ruling out Nitrogen addition

- Yeast suspensions of each species, let them sit for 8 hours
- Sent for YAN analysis at Iowa State’s Midwest Grape and Wine Industry Institute
  - Free Amino Nitrogen (for yeast growth)
  - Ammonium (Nitrogen available for plant growth)

FAN was low but present (between 5 – 37 mg/L)

*In wines, a healthy fermentation has >150 mg/L
No Ammonia/Ammonium

_We weren’t just spraying Nitrogen on the vines_
Spraying the yeast did something substantial!
  - Growers are engaged and interested in more research

But what did it do?
  - Move on to the more complicated
    - Hormonal shift?
    - Affecting gene expression?
Future Research Avenues

- Manipulating the microbiome with a specific goal in mind
- Using the microbiome to reduce pesticide applications
- Speeding up the process of microbiome data collection
Manipulating the microbiome with a goal in mind

- Can we encourage a microbial shift through management practices?
  - Cover crops
  - Sunlight exposure
  - Source-sink relationships
  - Livestock or silvopasture

- Or does the microbiome have to affected through directly applications?

- Can we use the microbiome to cut down on pesticide applications?
Using the microbiome to reduce pesticide applications

- No one wants to spray
- Can we get the microbiome to work to our advantage?
Using the microbiome to reduce pesticide applications

- “Bolstering” the microbiome
- Will an abundance of certain microbes prevent infection by certain pathogens?
- Insects deterred by some plant and microbial volatiles
  - Using this to our advantage
Thank you!

Questions?

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