Microbes of red raspberry buds, flowers, and fruit

Virginia Stockwell
Brenda Shaffer and Gayle McGhee
USDA-ARS
Horticultural Crops Research Unit
Corvallis, Oregon

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Characterizing the microbiome of developing floral tissues

Microbiome can be defined as the collection of microorganisms inhabiting an environment.

Morphogenesis from buds to flowers to fruit is a dynamic process.

Microbes must colonize and grow on changing tissues while exposed to a fluctuating conditions, such as rain, sun, temperature extremes, insect visitors, and other microbes competing for nutrients and sites for growth.
Methods to study the microbiome

• Molecular (culture independent) characterization
  • Grind tissues
  • Extract DNA
  • Use PCR to amplify universal genes for bacteria (16S) and fungi and yeasts (ITS region)
  • Sequences analyzed to identify genera present and their relative abundance

• Culture-based characterization
  • Mash tissues in buffer
  • Spread tissue washes and dilutions onto culture media
    • Tryptic soy agar with cycloheximide for bacteria
    • Potato dextrose agar with streptomycin for fungi and yeasts
  • Record populations and morphology of microorganisms recovered
  • Purify and store cultures, identify by morphology and molecular assays.
Field sites and sampling

Three ‘Meeker’ fields and two ‘Wakefield’ fields

Five replicate blocks per field

Ten samples per block

Stages sampled
Sample processing

Direct plating of samples

Surface disinfection before plating of samples
Average incidence of detection and population sizes on Meeker

**Site 1**

**Site 2**

**Site 3**

**Average incidence of detection**

**Mean Log$_{10}$ (colonies per tissue)**

- **Bacteria**
- **Fungi**
- **Yeasts**
Average incidence of detection and population sizes on Wakefield

Site 4

Site 5

Average incidence of detection

Bacteria

Fungi

Yeasts

Mean Log_{10}(colonies per tissue)
Bacteria present during full bloom across all five sites

Site 1- Meeker  Site 2- Meeker  Site 3- Meeker  Site 4- Wakefield  Site 5- Wakefield
Tracking of bacterial populations over time at site 3- Meeker

Bacteria: preliminary identification

- *Pantoea agglomerans*
- *Pseudomonas* spp.
- *Citrobacter* spp.
- *Raoultella* spp.
- *Rosenbergiella* spp.
- *Paenibacillus* spp.
- *Psychrobacillus* spp.
- {Uncultured bacterium}

Major late season:

- *Bacillus* spp.
Bacteria from surface disinfested fruit vs direct plated fruit

Bacteria reside mainly on the surface of ripe fruits.
Yeasts present during full bloom across five sites

Site 1-Meeker  Site 2-Meeker  Site 3-Meeker  Site 4-Wakefield  Site 5-Wakefield
Tracking yeast populations over time

Yeast: preliminary identification

*Cryptococcus albidosimilis*
*Cryptococcus saitoi*
*Cryptococcus victoriae*
*Sporobolomyces ruberrimus*

Major yeast present:

*Aureobasidium pullulans*
Yeasts from surface disinfested fruit vs Direct plated fruit

Site 5-Green Fruit

Yeasts reside mainly on the surface of fruits, not in internal tissues.
Tracking fungi across sites over time

Example of fungi isolated from tissues during prebloom

Fungi: preliminary identification

*Alternaria* spp.
*Botryotinia* spp.
*Botrytis* spp.
*Diaporthe* spp.
*Penicillium* spp.

Bud break  Pre bloom  Full bloom  Green fruit (surface sterilized)  Ripe fruit (surface sterilized)
Isolation of *Botrytis* spp.

*Botrytis* spp. was isolated from three of the five sites by pre-bloom and a fourth site by ~5 to 10% bloom.

The incidence of isolation of *Botrytis* spp. from pre-bloom and early bloom samples was low (ranging from 2 to 6%).

We did not determine if early season isolation of *Botrytis* spp. represented conidia that landed on tissues or if tissues were infected.
Mean incidence of storage rots by site

50 fruit from each block were surface disinfested and stored in humid containers. Incidence of fruit rot was monitored over 12 days.
Current findings

Microbial communities change over time and over different developmental stages of fruit formation.

By direct plating methods, we isolated the greatest diversity of bacteria, yeasts, and fungi from floral tissues.

We isolated bacteria (*Bacillus* spp), yeasts (*Aureobasidium* spp), and fungi from nearly every green fruit and ripe fruit. The diversity of the culturable microorganisms had decreased compared to bloom samples.

Studies of the microbiome may provide insight into the interactions between microorganisms and microbes with plant tissues that may influence the disease process.
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