Stop the Rot Onion Bacterial Project
Stakeholder Advisory Panel Meeting
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Objective A2b Microbiome Study

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https://alliumnet.com/projects/stop-the-rot/

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CMEG

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USDA United States Department of Agriculture National Institute of Food and Agriculture

Aims of the Study

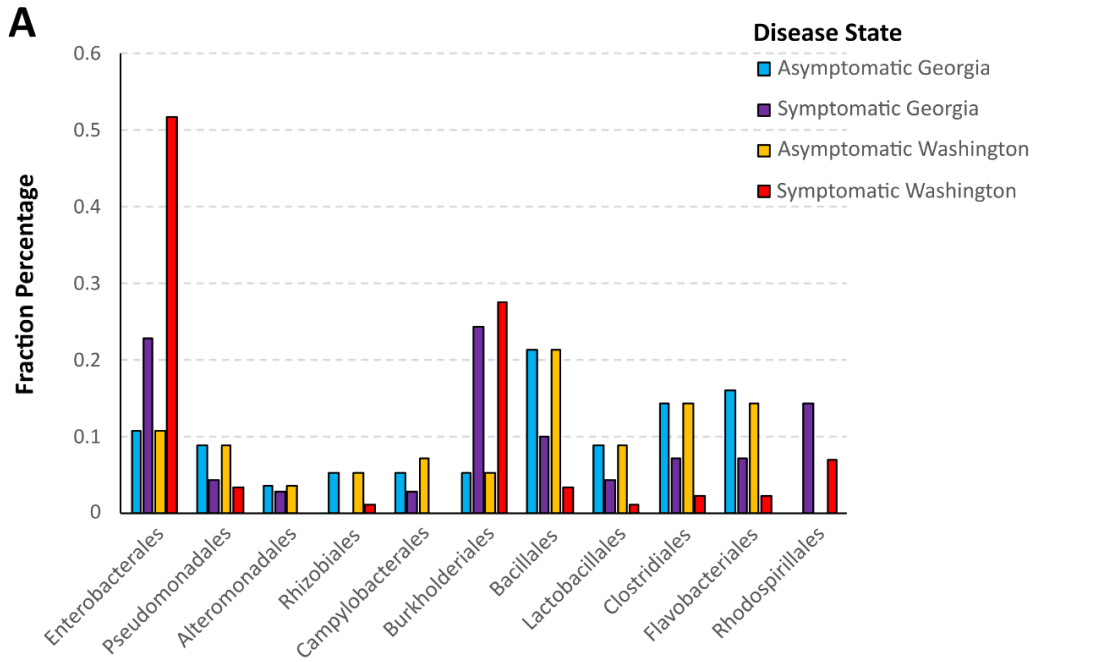
- Identify bacteria and viruses in storage onion bulbs symptomatic or asymptomatic for bacterial bulb rot, harvested from crops in Georgia and Washington
- Assess the potential role of the bacteria and viruses in bulbs based on functions of bacterial and viral genes detected

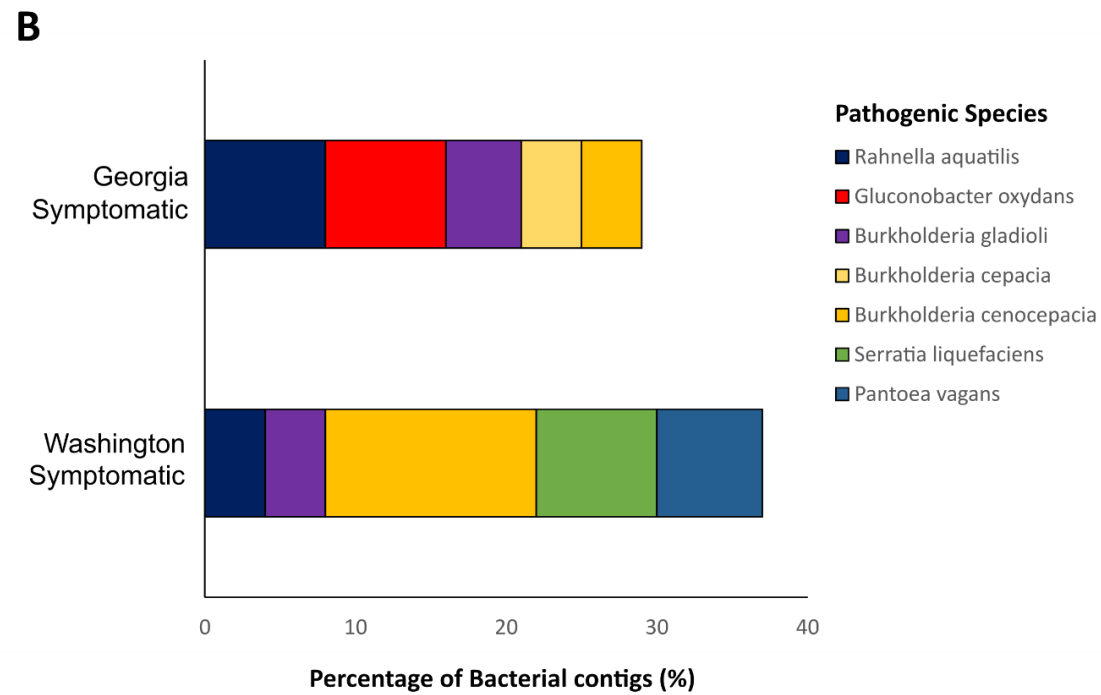
Methods

- DNA extracted from nine asymptomatic bulbs and nine bulbs with bacterial bulb rot symptoms
- 16S rRNA amplicon sequencing and Metagenomic Assembled Genome (MAG) analysis
- Illumina platform to sequence hypervariable region (V_3-V_4) of 16S rRNA gene

Results

- 16S rRNA amplicon profiling revealed numerous bacteria, including potential onion pathogens in *Panteoa* and *Burkholderia*
- MAG assembly identified *P. agglomerans*, *B. gladioli*, and *B. cepacia*, known bulb rot pathogens, including genes linked to fitness and genes involved in Type II and III secretion systems
- 89 unique viral genomes identified, of which 67 could be classified taxonomically
- Bacterial and viral genomes differed in asymptomatic vs. symptomatic bulbs
- Viral genomes showed evidence of auxiliary metabolic genes (AMGs) involved in fitness and pathogenicity to bacterial hosts (bacteriophages)


A
Disease State
Asymptomatic Georgia
Symptomatic Georgia
Asymptomatic Washington
Symptomatic Washington
Fraction Percentage
0.6
0.5
0.4
0.3
0.2
0.1
0
Enterobacterales
Pseudomonadales
Alteromonadales
Rhizobiales
Campylobacterales
Burkholderiales
Bacillales
Lactobacillales
Clostridiales
Flavobacteriales
Rhodospirillales


B
Pathogenic Species
Rahnella aquatilis
Gluconobacter oxydans
Burkholderia gladioli
Burkholderia cepacia
Burkholderia cenocepacia
Serratia liquefaciens
Pantoea vagans
Georgia Symptomatic
Washington Symptomatic
0
10
20
30
40
Percentage of Bacterial contigs (%)

Conclusions

- Onion bulbs were host to endophytic bacteria and viruses, some potentially beneficial, others potentially pathogenic to onion or hosts to bacteriophages
- Bacterial bulb rot may not be driven by a single pathogen, but may be influenced by co-habitation and interaction among bacteria
- Presence of bacteria in Acetobacterales in many symptomatic bulbs hints at a temporal dimension to progression of bacterial bulb rot
- Geographic location of onion crops in Georgia and Washington could have impacted primary bacterial pathogenic communities causing bulb rot, but geographic location was confounded with many other factors
- Viral populations in symptomatic onion bulbs could potentially be used as biocontrol agents
- Future studies: Assess the role of viral populations in bacterial rot progression in onion bulbs and, possibly, prevention
- **Questions/feedback from Stakeholder Advisory Panel?**