



Stop the Rot

Objective 2b

Microbiome analysis

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

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FABI



United States
Department of
Agriculture

National Institute
of Food and
Agriculture

Overview of Completed Research

1. In healthy onion bulbs and those displaying symptoms of rot we have completed:
 - 16S rRNA gene profiling
 - functional metagenomics
 - Virome analysis
2. Identified *Rahnella* species using MLSA
3. Characterized a bacteriophage capable of infecting *Pantoea ananatis*

16S rRNA gene profiling

- Members of the Burkholderiaceae and Acetobacteriaceae were abundant in diseased onion bulbs from Georgia
- Members of the Enterobacteriaceae were the most abundant in diseased onion bulbs from Washington State
- In healthy bulbs from Georgia, members of the Enterobacteriaceae were the most abundant
- Potential pathogens co-occur in the same diseased bulb

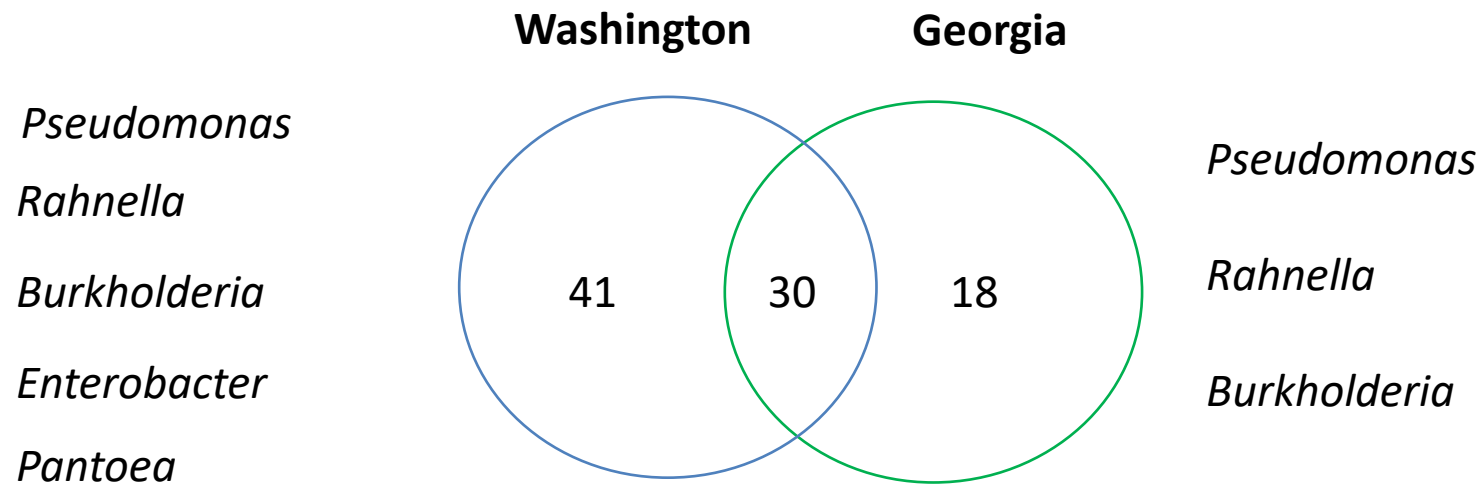


Functional Metagenomics

- Metagenome assembled genomes (MAGs) of known pathogens were derived from the diseased bulb datasets
- Included *Burkholderia gladioli* pv. *gladioli*, *Enterobacter ludwigii*, *Rahnella* spp. and *Pantoea agglomerans*
- No pathogens were detected in the healthy bulb datasets
- Focused on genes involved in the detoxification of the internal onion environment (e.g., glutaredoxin and thioredoxin), secreted pathogenicity elements (e.g., LysR family of transcriptional regulators) and secretion systems (e.g., Type III and IV)

Virome analysis

- High diversity of endophytic viruses in onion bulbs with bacterial rots
- Viral populations are associated with cultivars from different regions and target the host pathogens detected in these regions
- Endophytic viruses have marker genes that may help in host fitness and population control, i.e., biocontrol

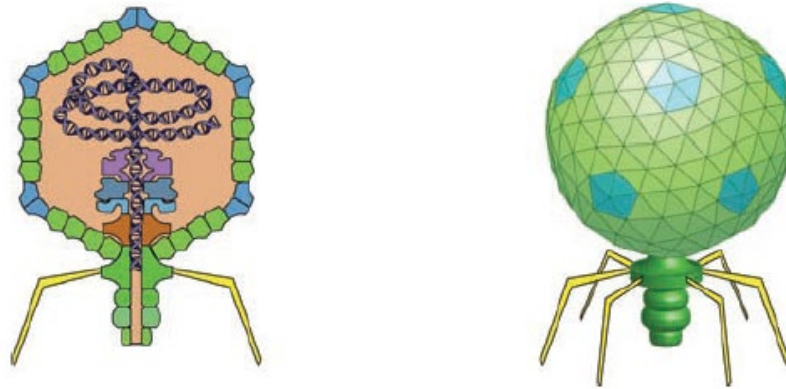


Identification of *Rahnella* spp.

- Identified seven species from diseased onion bulbs and leaves
- MLSA based on *rpoB*, *infB*, *gyrB* and *atpD* housekeeping genes
- Included *R. perminowiae* and *R. aceris* (most frequently isolated), *R. aquatilis*, *R. victoriana*, *R. variigena*, *R. rivi* and *R. bonaserana*

Characterization of a Bacteriophage

- Purified, sequenced and characterized a bacteriophage (in the family *Autographiviridae*)
- Novel strain similar to ones isolated in South Korea for control of *Erwinia pyrifoliae*
- Bacteriophage is lytic and capable of infecting *P. ananatis* (strain 97-1R)
- Does not infect *P. agglomerans* or any other bacteria capable of causing bulb rot



Preliminary plans for Session 3

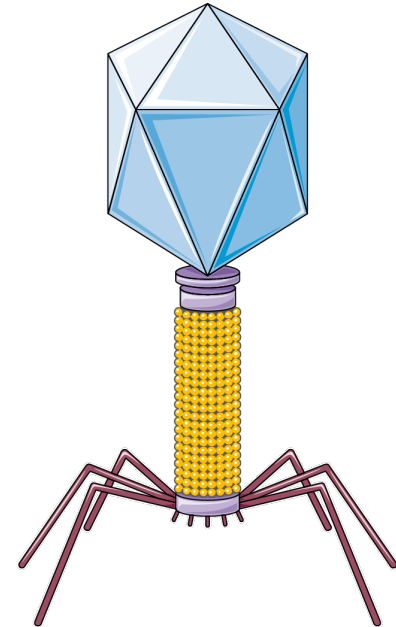
- Complete the functional metagenome analysis of the onion bulb datasets from Georgia focusing on metabolic pathways present in the MAGs
- Submit a MSc dissertation for examination (April 2022)
- Prepare the manuscript for publication (include the 16S rRNA profiling, functional metagenomics and the virome analysis)

Preliminary plans for Session 3

- *In planta* infection assays with the different *Rahnella* spp.
- Prepare manuscript for publication (2022)
- Select strains from *R. perminowiae* for genome sequencing
- Comparative genomics, ideally of strains pathogenic and non-pathogenic on onion
- Identify pathogenicity factors
- MLSA and MALDI-TOF of *Enterobacter* strains

Preliminary plans for Session 3

- Search for bacteriophages infecting more than one *Pantoea* spp. and those infecting other bulb rot pathogens
- Sequence and characterize the bacteriophages
- Undertake *in planta* trials
- Submit a MSc dissertation (Oct. 2023)
- Prepare a manuscript for publication



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