

A study on bacterial inhabitants of storage onion bulbs: identity and function

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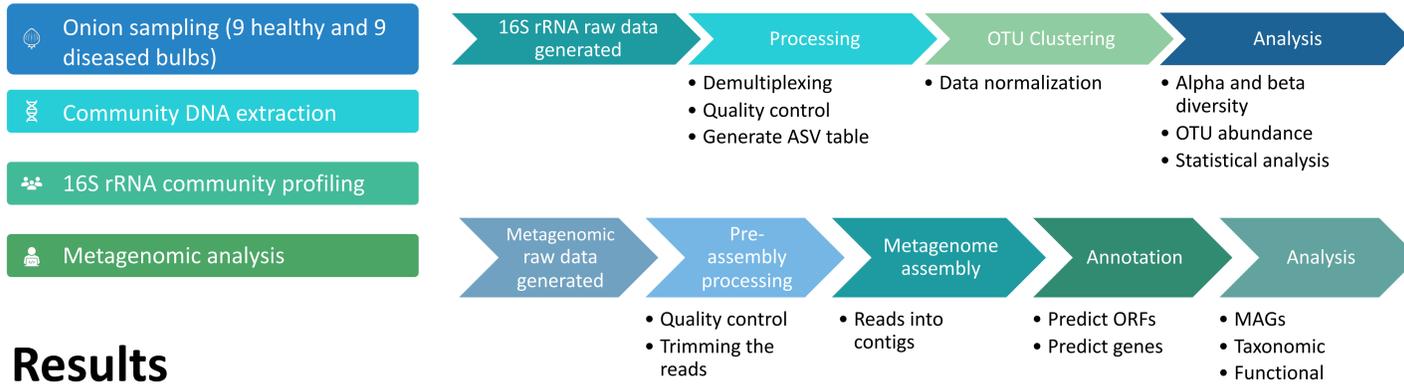
Introduction

Within different parts of a plant, microbes occur and establish a network of interactions that influences their health, either positively or negatively. Some bacterial species found in association with plants can switch their lifestyle from non-pathogenic to pathogenic, causing serious diseases that have major impacts on crop production. In the case of onion bulb rot, a complex of pathogenic bacteria may occur in the same bulb together with non-pathogenic bacteria, including the same species as those known to cause bulb decay. The trigger for the change from a non-pathogenic to a pathogenic state is not well understood.

Aims & Objectives

The aim of this study was to determine the taxonomic composition of bacteria and their function in healthy bulbs as well as bulbs displaying symptoms of bacterial bulb rots. Bulbs were collected from storage facilities in Georgia and Washington States. The bacterial community within the bulbs was identified by sequencing of the V3-V4 region of the 16S rRNA gene region using a MiSeq Illumina sequencing platform. Metagenome assembled genomes (MAGs) were identified and their function predicted.

Methods



Results

16S rRNA community profiling

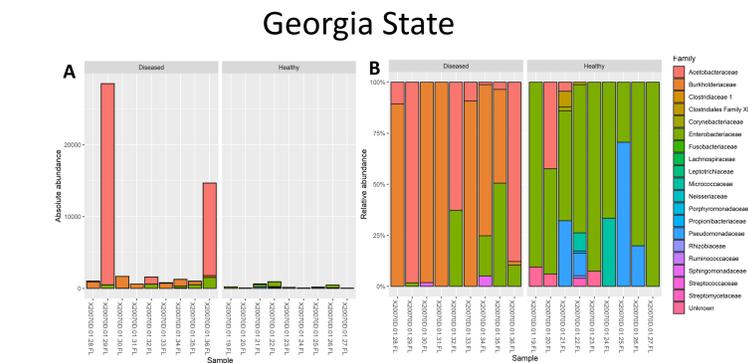


Fig 1 [A] Absolute abundance of all 16S rRNA reads [B] Relative abundance of 16S rRNA reads, excluding mitochondrial and chloroplast reads, per onion bulb from Georgia State. The taxonomic composition shows two distinct structures present in the healthy and diseased bulbs. Members of the Enterobacteriaceae were dominant in the healthy bulbs while Burkholderiaceae and Acetobacteriaceae were dominant in the diseased bulbs.

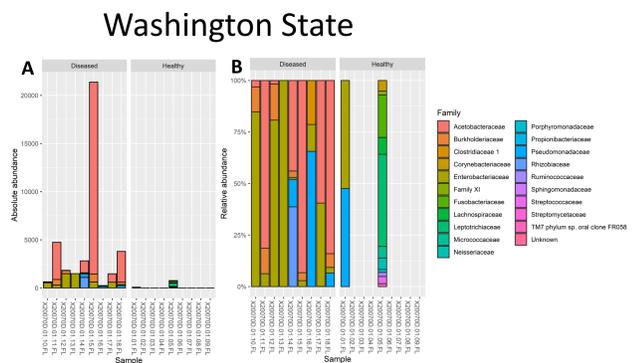


Fig 2 [A] Absolute abundance of all 16S rRNA reads [B] Relative abundance of 16S rRNA reads, excluding mitochondrial and chloroplast reads, per onion bulb sample from Washington State. In the diseased bulbs, several genera were present, with members of Enterobacteriaceae found in all nine bulbs, *Burkholderia* and *Gluconobacter* in six bulbs, *Pseudomonas* in three, and *Clostridium*, *Gluconacetobacter*, *Ochrobactrum*, and *Kosakonia* each in one bulb only.

Conclusion

In the onion bulbs from Georgia with bacterial rot, Enterobacteriaceae, Acetobacteriaceae and Burkholderiaceae were dominant, and there were notably more species detected in the healthy bulbs compared to healthy bulbs sampled in Washington State. In Washington, several potentially pathogenic members of the Enterobacteriaceae and Burkholderiaceae were present in symptomatic bulbs, including *Burkholderia*, *Pantoea*, and *Pseudomonas*, with some co-existing within the same bulb, indicating possible polymicrobial infections. From the metagenomic data, *Burkholderia* spp. and *Pseudomonas* spp. had the most potential to cause bulb rots (based on the presence of known pathogenicity factors), with *Enterobacter*, *Pantoea* and fermenter genera (*Gluconacetobacter* and *Acetobacter*) likely exacerbating onion necrosis and decay. In both states, increased biofilm formation activity, bacterial chemotaxis, secretion systems and sulfur metabolism were detected only in the bulbs with bacterial rot symptoms.

Metagenomic bioinformatic analysis

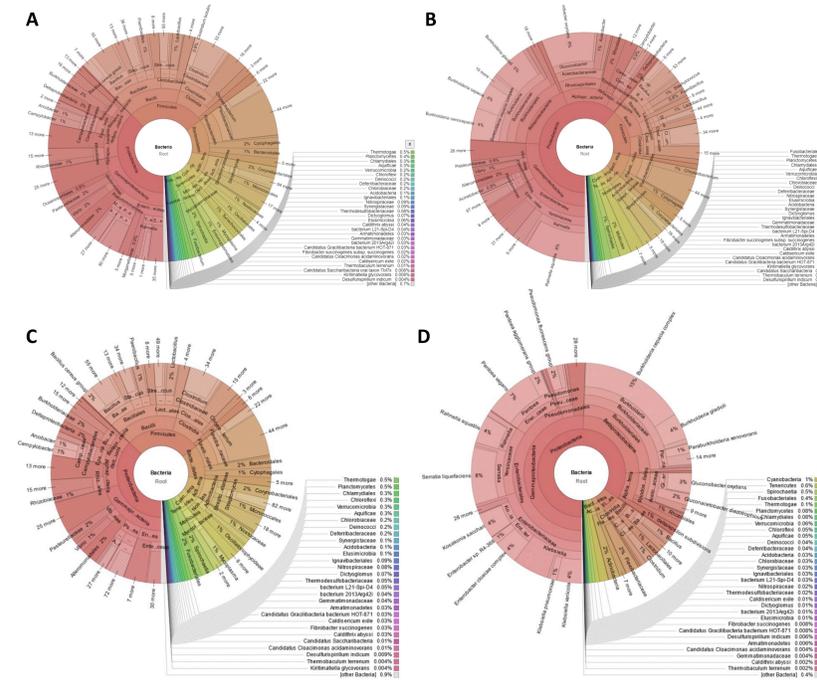


Fig 3 Krona graphs indicating the taxonomy as well as the relative abundance of bacterial reads from [A] healthy and [B] diseased onion bulbs sampled in Georgia State, and reads from [C] healthy and [D] diseased onion bulbs sampled in Washington State.

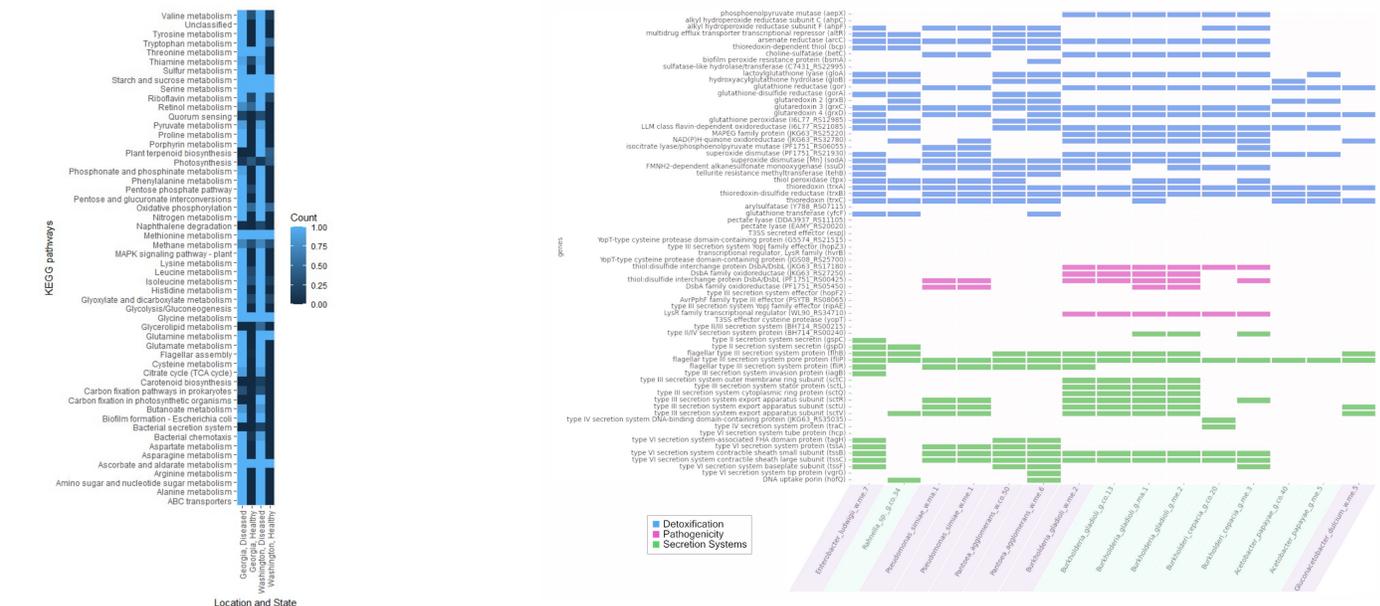


Fig 4 Heatmap showing the pathways in onion bulbs sampled from Georgia and Washington States, generated by KofamKHOALA using an HMM profile search that assigns KEGG ontology numbers to each pathway.

Fig 5 Presence or absence of select genes from MAGs in onion bulbs with bacterial rot symptoms sampled from Washington and Georgia.

Taxonomic composition

The Krona graphs indicate the taxonomy as well as the relative abundance of bacteria in the reads of healthy and diseased onion bulbs from Georgia and Washington State s (Fig 3).

The metagenomic data trend was similar to the 16S rRNA communities (Figs 1 & 2). In Georgia bulbs, members of the Enterobacteriaceae, Acetobacteriaceae and Burkholderiaceae were dominant in the diseased bulbs while members of the Enterobacteriaceae was dominant in the healthy onion bulbs.

The metagenome assembled genomes (MAGs) in Georgia included: *Acetobacter papaya*, *Burkholderia cepacia*, *B. gladioli* and *Rahnella* spp., while Washington bulbs included *B. gladioli*, *Enterobacter ludwigii*, *Gluconacetobacter dulcium*, *Pantoea agglomerans* and *Pseudomonas simiae*.

Functional assessment

The diseased onion bulbs from Georgia and Washington had greater activity in amino acid metabolism, quorum sensing, flagellar assembly, biofilm formation, bacterial secretion systems and chemotaxis compared to healthy bulbs. This indicates that the MAGs in the diseased bulbs had more metabolically active genes with known pathogenicity factors (Figs 4 & 5).

Acknowledgements

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