

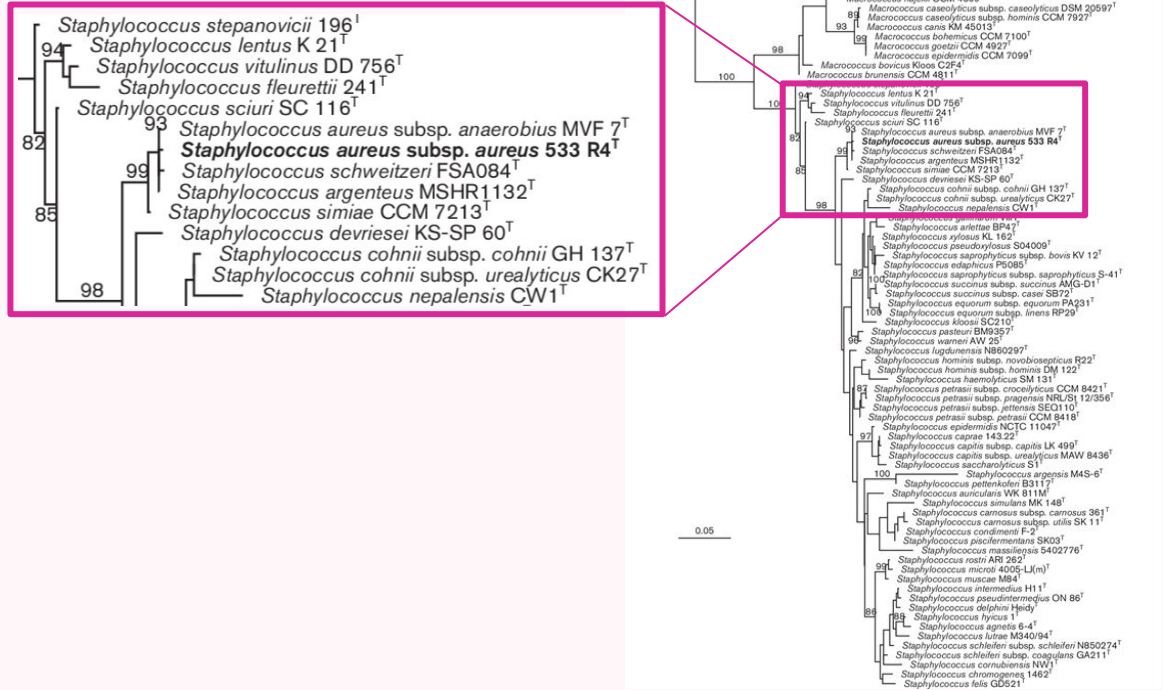
Virulence Factors of Mammiliicoccus

Ashley Rosborough

Background

Mammiliicoccus

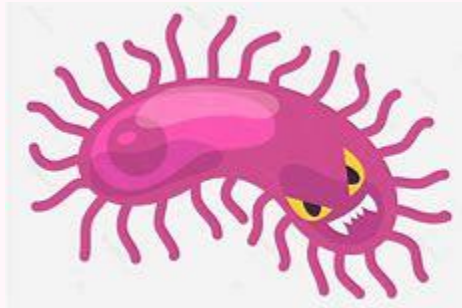
- Sister genus of *Staphylococcus* gena
- Moved following 16s RNA gene similarity analysis
- Gram positive cocci
- Non-motile
- Non-spore forming



Mammiliicoccus and Disease

Non-*aureus* staphylococci and mammaliicocci (NASM)

- Lack of coagulase virulence factor
- Opportunistic pathogens
 - Mastitis in dairy animals
 - *M. sciuri* - ExhC Exfoliation and necrosis
 - Severe Exudative Epidermitis pig outbreak in China
 - Endocarditis, peritonitis, septic shock, UTI, PID, wound infections



Goal

High similarity to *Staphylococcus aureus*

- Perform assembly of *Mammiliicoccus* genome
- Identify and compare virulence factors
- Highlight concerns with mushroom compost mediums

Sample Isolation

Anand Karki, Ph.D.

2015-2017 - Mushroom isolates cultured and identified

2020 - Illumina Miseq short reads

2022-2023 - Oxford Nanopore long reads

2024 - Got my hands on them

Trimming - Trimmomatic

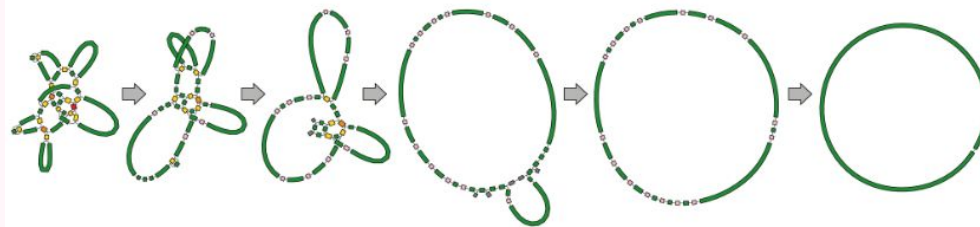
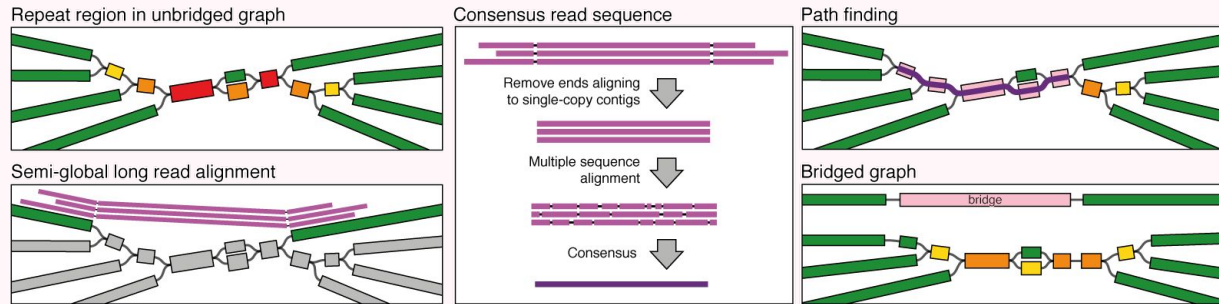
Illumina short reads

- Trimmed via Trimmomatic
 - Available adapters moved into master list
 - Removed:
 - ILLUMINACLIP - Adaptors
 - LEADING:3 - Low leading quality or N bases
 - TRAILING:3 - low trailing quality or N bases
 - SLIDINGWINDOW:4:15 - 4 b wide cut if average quality per base below 15
 - MINLEN:36 - Reads below 36 b in length

Assembly - Unicycler

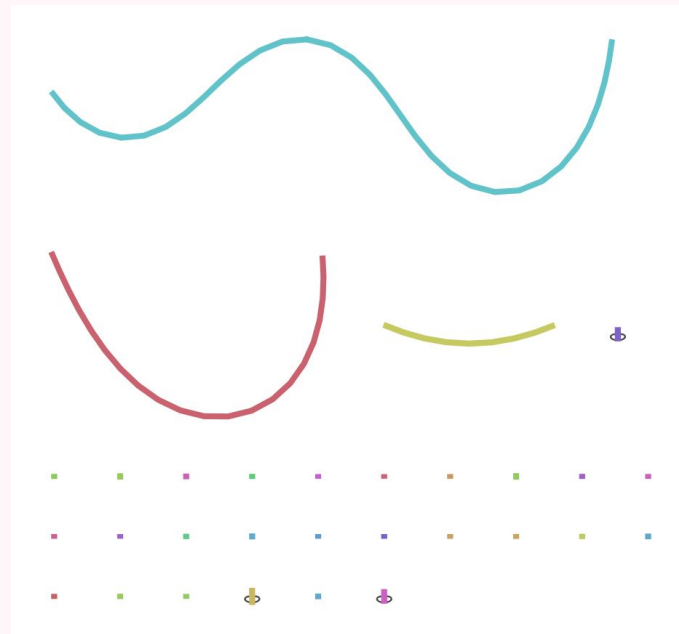
Unicycler hybrid assembly

- Repeats longer than the read - form singular contig
- Uses SPAdes to produce de Bruijn graph
 - Different k -mer lengths - increased connections and repeat resolutions

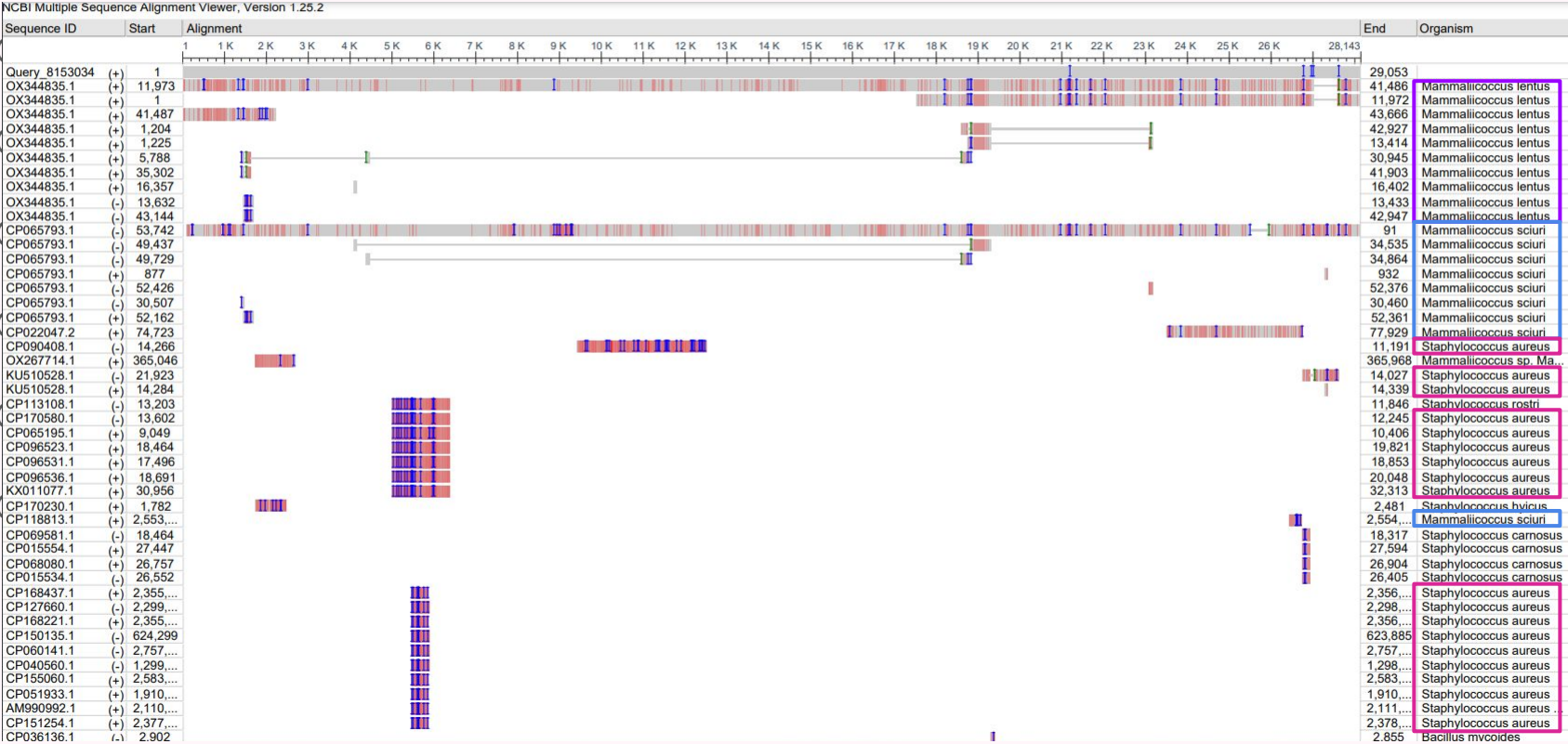


Bandage

Visualizes .gfa files



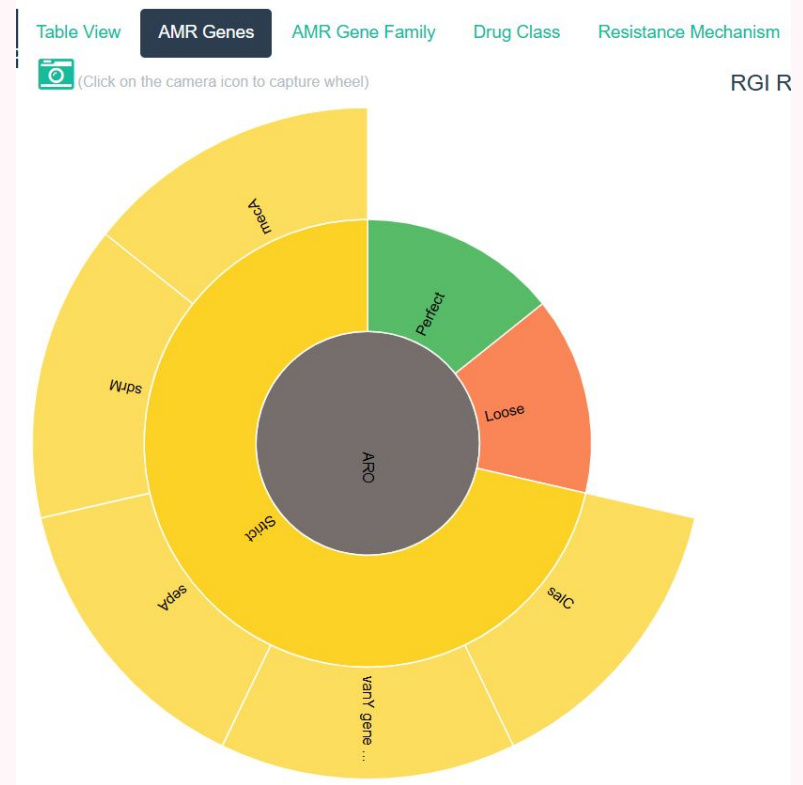
BLAST



CARD RGI Resistance Gene Identifier

Separate contigs with circular=true

Generates .json and .txt files



CARD cont.

Mammaliicoccus vitulinus

bc03 and bc05 core genome

- Glycopeptide
 - Antibiotic target alteration
- Lincosamide, streptogramin, streptogramin A, pleuromutilin
 - Antibiotic target protection
- Fluoroquinolone, disinfecting agents and antiseptics
 - Major facilitator superfamily (MFS) antibiotic efflux pump
- Disinfecting agents and antiseptics
 - Small multidrug resistant (SMR) antibiotic efflux pump
- Penam
 - Antibiotic target replacement
 - Methicillin resistant

bco3 plasmid

- Disinfecting agents and antiseptics
 - SMR antibiotic efflux pump

Card cont.

Staphylococcus xylosus

bc04 core genome

- Glycopeptide x2
- Fluoroquinolone, disinfecting agents and antiseptics x2
 - MFS efflux
- Disinfecting agents and antiseptics
 - SMR efflux
- Phosphonic acid antibiotic
 - Antibiotic target alteration

bc04 plasmid

- Disinfecting agents and antiseptics
 - SMR efflux

bc06 core genome

- Glycopeptide x3
- Lincosamide, streptogramin, streptogramin A, pleuromutilin
 - Antibiotic target protection
- fluoroquinolone, disinfecting agents and antiseptics x3
 - MFS
- Disinfecting agents and antiseptics
 - SMR efflux

bc06 plasmid

- none

Card cont.

Staphylococcus pasterui

bc07 core genome

- Glycopeptide
 - Antibiotic target alteration
- Fluoroquinolone x2, aminoglycoside, penam, tetracycline, disinfecting agents and antiseptics x2
 - MFS
- Phosphonic acid
 - Antibiotic inactivation
 - Fosfomycin thiol transferase
- Aminocoumarin
 - Antibiotic target alteration

bc06 plasmid

- Penam
 - Antibiotic inactivation
 - beta-lactamase

Card cont.

Staphylococcus warneri

bc08 core genome and 9

- Glycopeptide x3
 - Antibiotic target alteration
- Fluoroquinolone, disinfecting agents and antiseptics
 - MFS
- Disinfecting agents and antiseptics
 - SMR
- Aminocoumarin
 - Antibiotic target alteration

bc08 and bc09 plasmid

- Macrolide, streptogramin, streptogramin B antibiotic
 - Antibiotic target protection
- Penam
 - Antibiotic inactivation
 - Beta-lactamase
- Fluoroquinolone, aminoglycoside, penam, tetracycline, disinfecting agents and antiseptics
 - MFS

bc09 core genome and 9

- Glycopeptide x2
 - Antibiotic target alteration
- Fluoroquinolone, disinfecting agents and antiseptics
 - MFS
- Disinfecting agents and antiseptics
 - SMR
- Aminocoumarin
 - Antibiotic target alteration

Card cont.

Staphylococcus haemolyticus

bc10 core genome

- Glycopeptide x2
 - Antibiotic target alteration
- Fluoroquinolone, disinfecting agents and antiseptics x3
 - MFS
- Disinfecting agents and antiseptics
 - SMR

bc10 plasmid

- Macrolide, streptogramin, streptogramin B
 - Antibiotic target protection
- Macrolide antibiotic
 - Macrolide phosphotransferase

Virulence Factor of Pathogenic Bacteria (VFPD)

DNA sequences from full dataset

| Sequences producing significant alignments: | | Score (bits) | E Value |
|---|--|--------------------|------------|
| VFG032615 | (lisR) response regulator transcription factor [L... | 84 | 2e-12 |
| VFG019394 | (lisR) response regulator transcription factor [L... | 76 | 6e-10 |
| VFG048968 | (gndA) NADP-dependent phosphogluconate dehydrogen... | 76 | 6e-10 |
| VFG047717 | (carB) carbamoyl phosphate synthase large subunit... | 70 | 3e-08 |
| VFG032626 | (lisR) response regulator transcription factor [L... | 68 | 1e-07 |
| VFG032624 | (lisR) response regulator transcription factor [L... | 68 | 1e-07 |
| VFG032607 | (lisR) response regulator transcription factor [L... | 68 | 1e-07 |
| VFG032604 | (lisR) response regulator transcription factor [L... | 68 | 1e-07 |
| VFG019393 | (lisR) response regulator transcription factor [L... | 68 | 1e-07 |
| VFG006827 | (lisR) response regulator transcription factor [L... | 68 | 1e-07 |

Future Directions

- Finish analyzing resistances
- Find way to identify and locate virulence factors
- Merge remaining contigs - CLC
- Analysis comparing virulence genes between my samples and *S. aureus*

Resources

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Suggest the Reclassification of Five Species within the Genus *Staphylococcus* as Heterotypic Synonyms, the Promotion of Five Subspecies to Novel Species, the Taxonomic Reassignment of Five *Staphylococcus* Species to *Mammaliicoccus* Gen. Nov., and the Formal Assignment of *Nosocomiicoccus* to the Family Staphylococcaceae." *Microbiology Research*, Microbiology Society, 14 Oct. 2020, www.microbiologyresearch.org/content/journal/ijsem/10.1099/ijsem.0.004498#tab2.

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Usadellab, BjoernUsadel. "Trimmomatic." GitHub, github.com/usadellab/Trimmomatic.

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Thank you!

Any questions?

