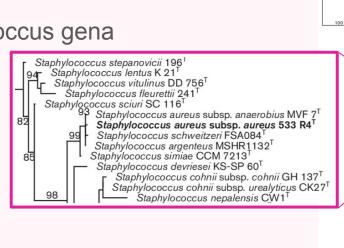
Virulence Factors of Mammiliicoccus

Ashley Rosborough

Background

Mammiliicoccus

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



Abyssicoccus albus DSM 29158 Auricoccus indicus S31

Allococus persicus (BRC-M10081* |
100 Nessocomicocus ampullea TRF-1* |
100 Nessococus perspectable (Nessococus perspectable (Nessococus perspectable) (Nessococus perspectable (Nessococus perspectable) (Nessococus perspectable (Nessococus perspectable) (Nessoco

Salinicoccus halodurans CGMCC 1.6501^T
Salinicoccus carnicancri Crm^T

Indignation and the state of th

Salinicoccus qingdaonensis ZXM223¹
Corticicoccus populi 26D10-3-4¹

Macrococcus bohemicus CCM 7100 Macrococcus goetzii CCM 4927

Staphylococcus aureus subsp. aureus 533 R4
Staphylococcus schweitzer FSA084

Staphylococcus cohnii subsp. cohnii GH 137^T Staphylococcus cohnii subsp. urealyticus CK27 Staphylococcus nepalensis CW1^T

Staphylococcus pseudoxylosus S04009^T

Staphylococcus equorum subsp. linens RP29

Staphylococcus kloosii SC210

 Staphylococcus saprophyticus subsp. bovis KV 12^T Staphylococcus edaphicus P5085^T

Staphylococcus saprophyticus subsp. saprophyticus S-41
Staphylococcus succinus subsp. succinus AMG-D1
Staphylococcus succinus subsp. casei SB72

Staphylococcus argenteus MSHR1132^T

Staphylococcus arlettae BP47
 Staphylococcus xylosus KL 162

taphylococcus simiae CCM 7213[†] Staphylococcus devriesei KS-SP 60[†]

Staphylococcus pasteuri BM9357 Staphylococcus warneri AW 25¹ Staphylococcus lugdunensis N860297 Staphylococcus hominis subsp. novobiosepticus R22¹ Staphylococcus hominis subsp. hominis DM 122¹ Staphylococcus haemolyticus SM 131 87 Staphylococcus petrasii subsp. croceilyticus CCM 8421¹ Staphylococcus petrasii subsp. pragensis NRL/St 12/356¹ Staphylococcus petrasii subsp. jettensis SEQ110[†] Staphylococcus petrasii subsp. petrasii CCM 8418[†] Staphylococcus epidermidis NCTC 11047[†] Staphylococcus caprae 143.22 Staphylococcus capitis subsp. capitis LK 499^T Staphylococcus capitis subsp. capitis LK 499'
Staphylococcus sacris subsp. urealyticus MAW 8436'
Staphylococcus saccharolyticus S1'
Staphylococcus argensis M4S-6'
Staphylococcus pettenkoleri B3117' Staphylococcus auricularis WK 811M¹
 Staphylococcus simulans MK 148¹ Staphylococcus carnosus subsp. carnosus 361 Staphylococcus carnosus subsp. utilis SK 11¹ Staphylococcus condimenti F-2¹ Staphylococcus piscifermentans SK031 Staphylococcus massiliensis 5402776^T Staphylococcus rostri ARI 2621 Staphylococcus microti 4005-LJ(m) Staphylococcus muscae M84 Staphylococcus intermedius H111 Staphylococcus pseudintermedius ON 86 Staphylococcus delphini Heidy Staphylococcus hyicus 1 Staphylococcus agnetis 6-4^T
Staphylococcus lutrae M340/94^T
Staphylococcus schleiferi subsp. schleiferi N850274^T Staphylococcus schleiferi subsp. coagulans GA211 - Staphylococcus cornubiensis NW1 Staphylococcus chromogenes 1462[†] Staphylococcus felis GD521 [†]

Macrococcus epidermidis CCM 7099

Macrococcus bovicus Kloos C2F4

Macrococcus bovicus Kloos C2F4

Macrococcus brunensis CCM 4811

Staphylococcus vitulinus DD 756^T
Staphylococcus fleuretii 241^T
Staphylococcus Staphylococcus aureus subsp. anaerobius MVF 7^T

Macrococcus caseolyticus subsp. caseolyticus DSM 20597^T
Macrococcus caseolyticus subsp. hominis CCM 7927^T
Macrococcus canis KM 45013^T

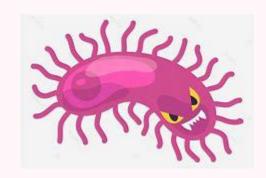
Salinicoccus alkaliphilus T8[†]
Salinicoccus halififaciens JC90[†]
"Salinicoccus kensnis" K164[†]
Salinicoccus kunmingensis YIM Y15[†]
Salinicoccus kunmingensis YIM Y15[†]
Salinicoccus albus YIM-Y21[†]

Macrococcus carouselicus DD 9348¹
Macrococcus equipercicus DD 9350[†]
Macrococcus lamae CCM 4815[†]
Macrococcus haiekii CCM 4809[†]

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

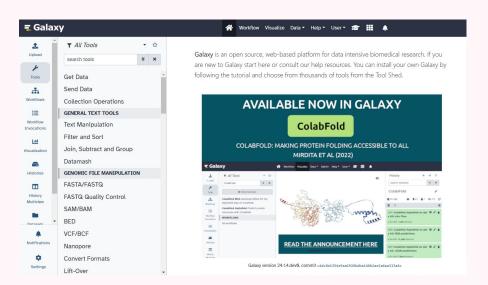
Illumina short reads

- 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

Trimming - Porechop

Oxford long reads

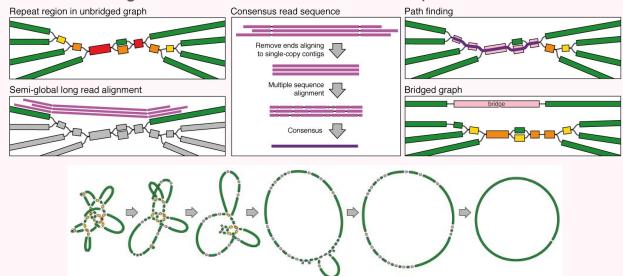
- Trimmed via Galaxy's Porechop
 - First and last bases aligned to adapter set
 - If over 90% adapter match, read trimmed
 - If match in middle of read, read is split
- Concatenated



Assembly - Unicycler

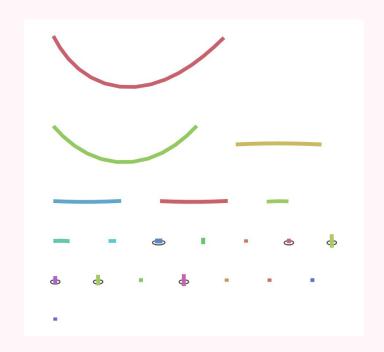
Unicycler hybrid assembly

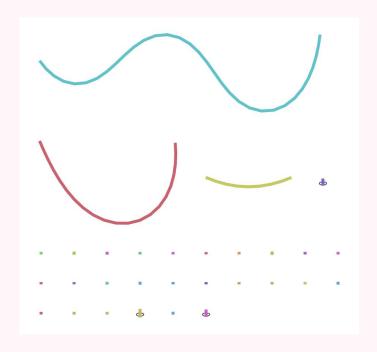
- Repeats longer than the read form singular contig
- Uses SPAdes to produce de Bruijn graph
 - Different *k*-mer lengths increased connections and repeat resolutions



Bandage

Visualizes .gfa files



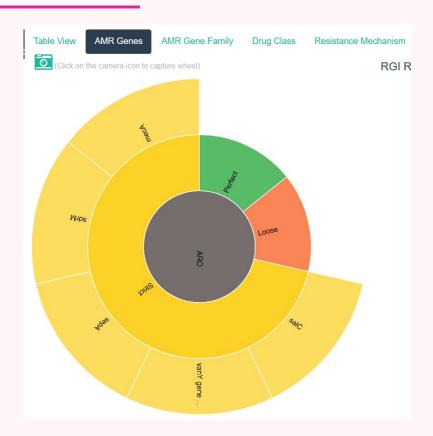


BLAST



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
- Fluoroguinolone, 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

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

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 transferace
- Aminocoumarin
 - Antibiotic target alteration

bc06 plasmid

- Penam
 - Antibiotic inactivation
 - beta-lactamase

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

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

```
Score
                                                                         F
                                                                 (bits) Value
Sequences producing significant alignments:
          (lisR) response regulator transcription factor [L...
VFG032615
                                                                       2e-12
VFG019394
           (lisR) response regulator transcription factor [L...
                                                                  76
                                                                       6e-10
VFG048968
          (gndA) NADP-dependent phosphogluconate dehydrogen...
                                                                       6e-10
                                                                 76
          (carB) carbamoyl phosphate synthase large subunit...
                                                                       3e-08
VFG047717
                                                                 70
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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- Wick, Ryan. "Unicycler: Hybrid Assembly Pipeline for Bacterial Genomes." GitHub, github.com/rrwick/Unicycler.

Thank you!

Any questions?

