Tracking the Source of Fecal Contamination to Western Galveston Bay

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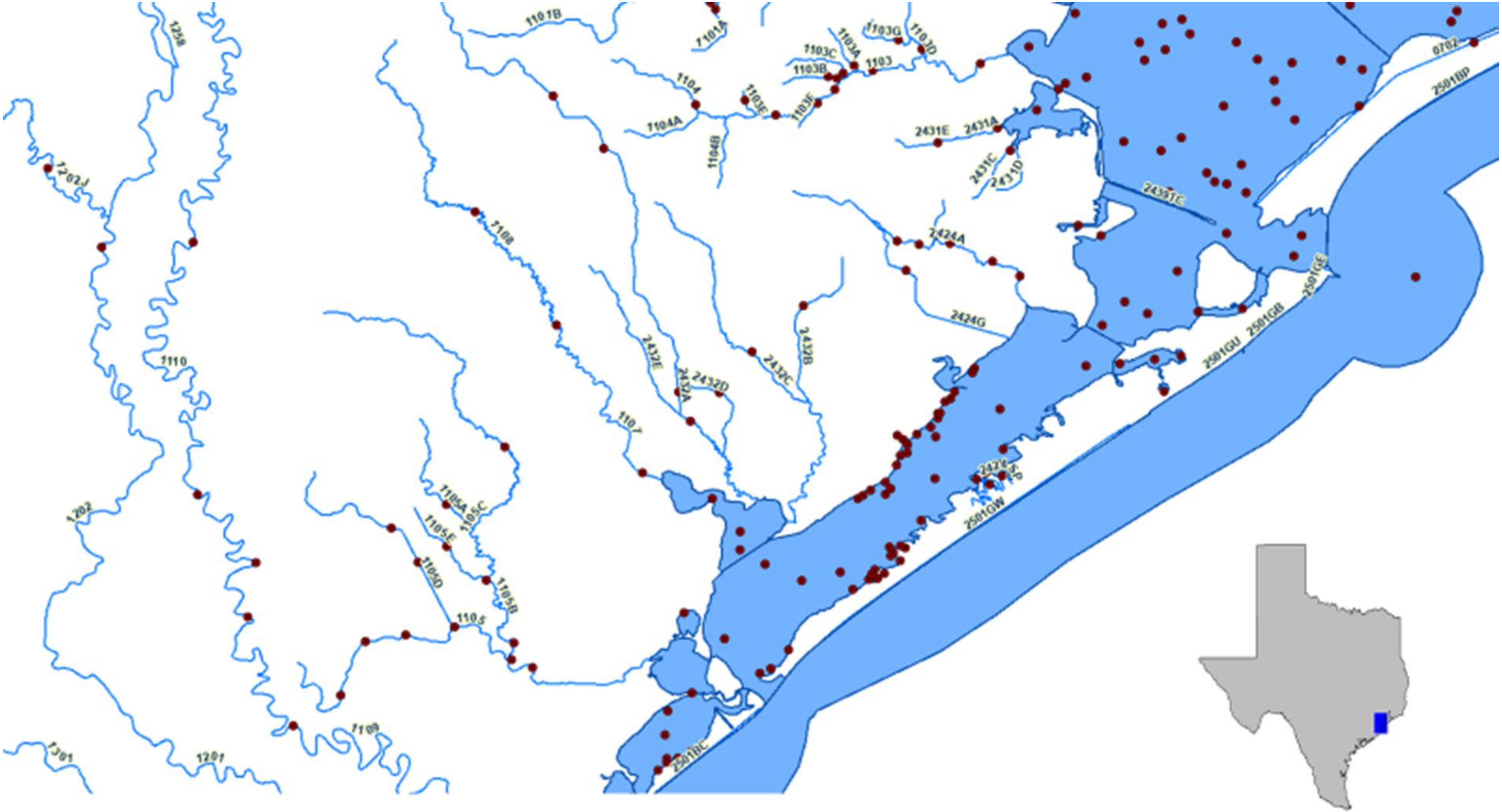
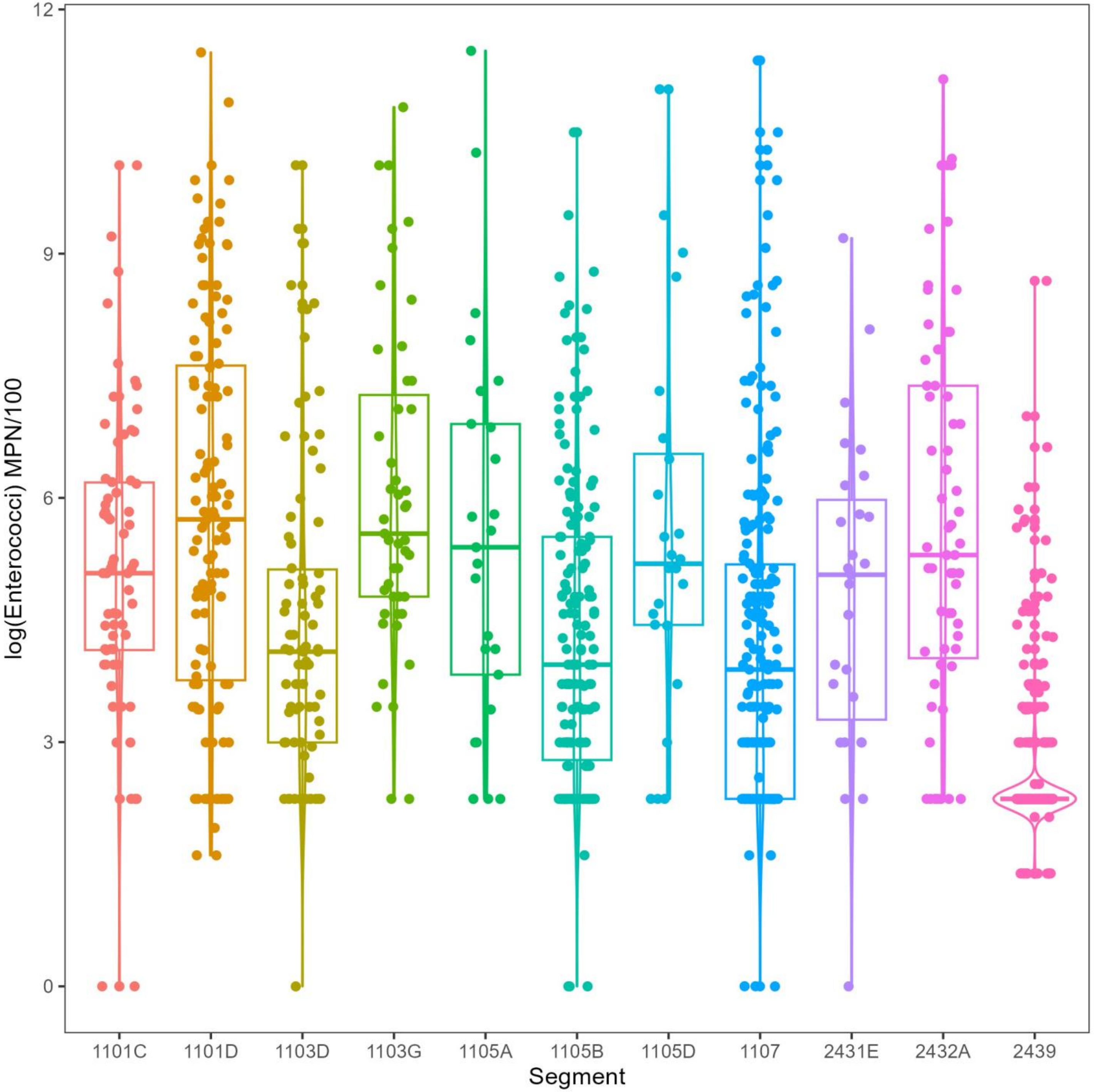
Microbial Source Tracking

- Waterways receive fecal contamination from diverse sources
- Human waste contamination presents acute public health risk

Vadde (2019) Front Micro


1101B
1101A
1103G
1103D
1103A
1103C
1103B
1103
1104
1103E
1103F
1104A
1104B
2431E
2431A
2431C
2431D
2438TC
0702
2501BP
1258
1202J
1108
2424A
2424G
2501GE
2501GB
2501GU
2432E
2432A
2432D
2432C
2432B
1110
1107
2424
SP
2501GW
1105A
1105C
1105E
1105D
1105B
1105
1202
1109
1301
1201
2501BC



log(Enterococci) MPN/100
12
9
6
3
0
1101C
1101D
1103D
1103G
1105A
1105B
1105D
1107
2431E
2432A
2439
Segment

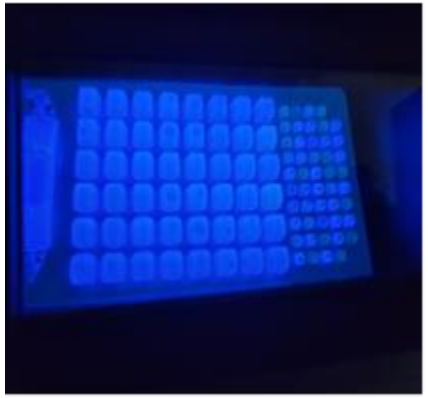
Conceptual Model

- Metagenomic
- Culture/library-dependent

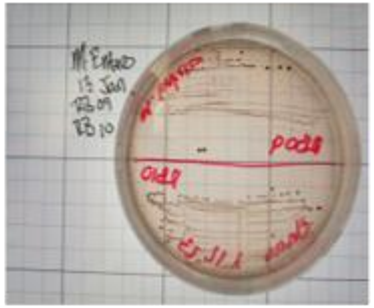
Water sample

Concentrating pippette

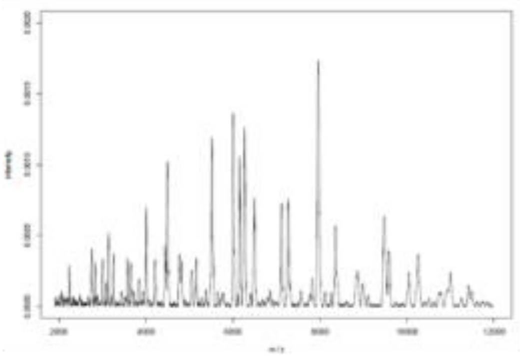
dPCR



IDEXX Enterolert



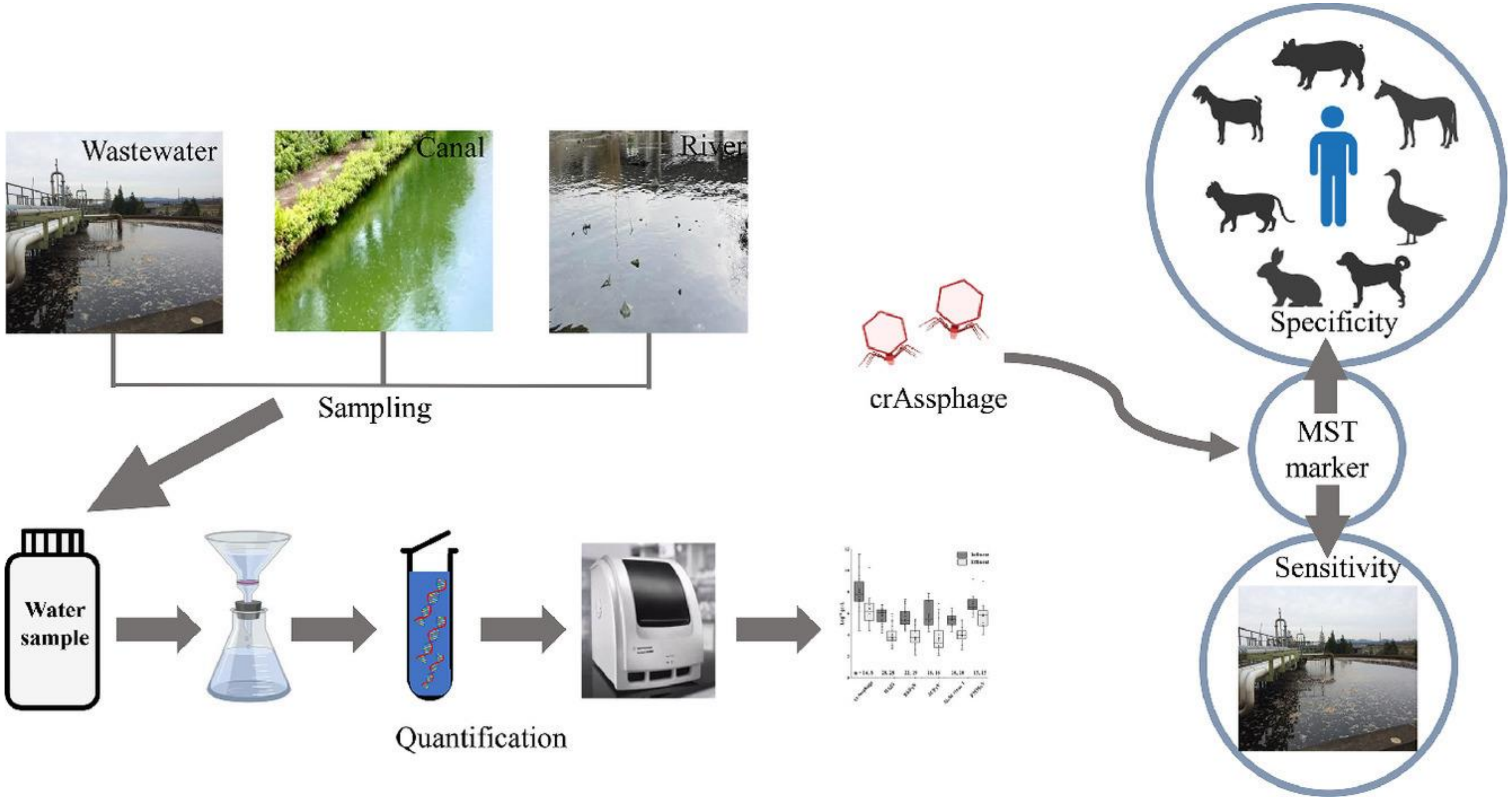
Differential and selective media



Mass spectra

crAssphage

- bacteriophage found in high concentrations in human waste
- Emerging tool for tracking human waste in receiving waters

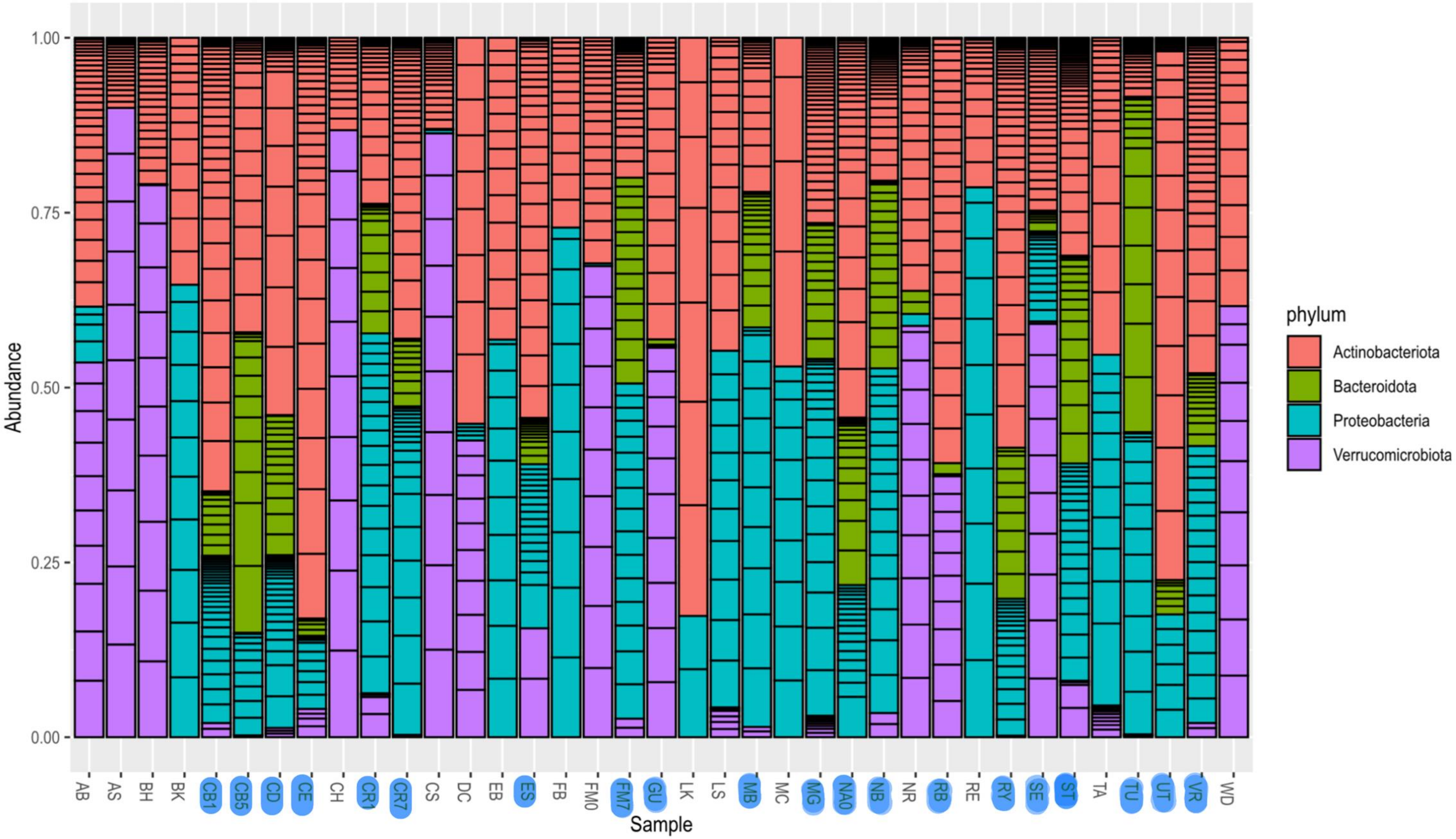


Sabar (2022) https://doi.org/10.1016/j.watres.2022.118827

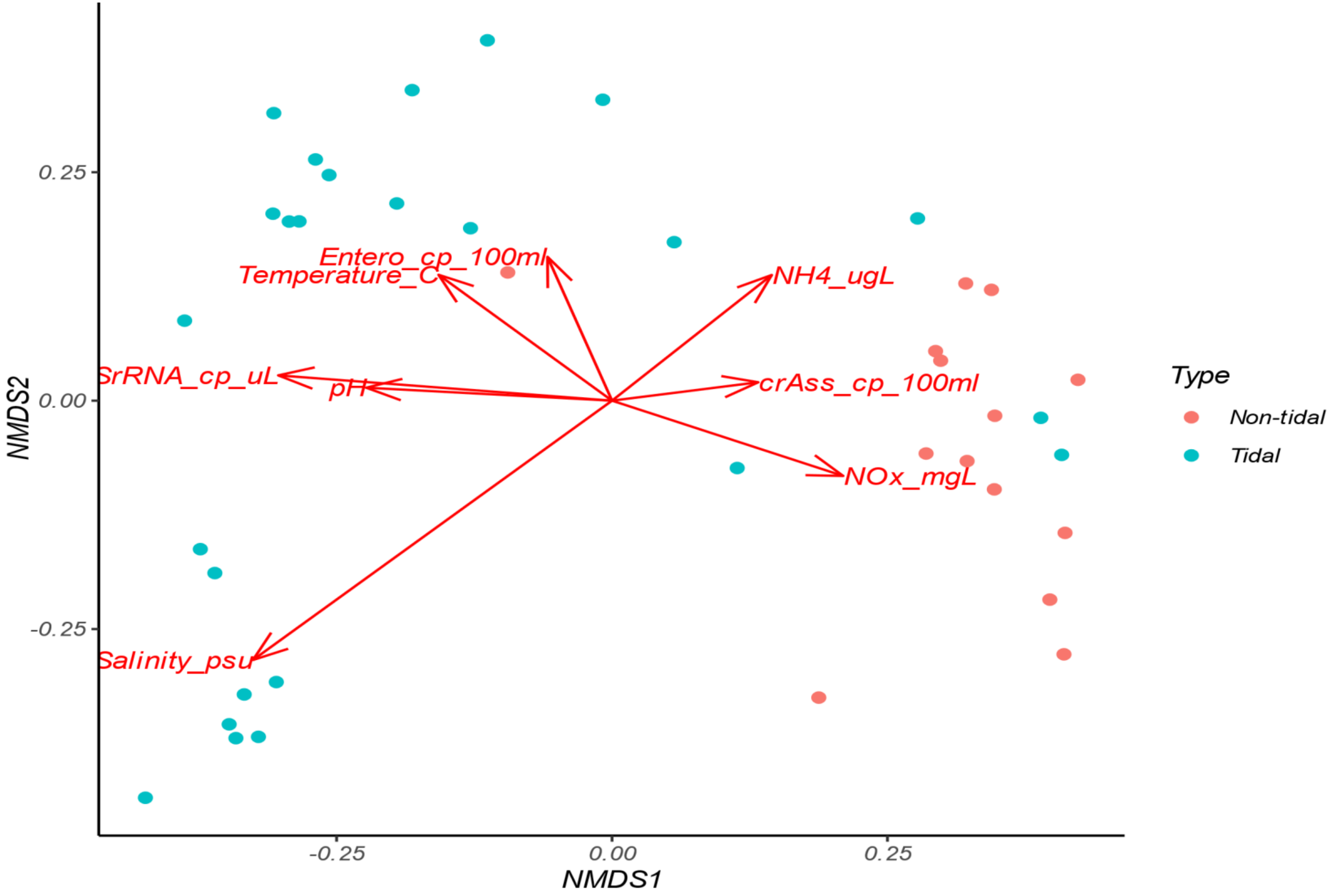
Metagenomic Methods

- Stations in tributaries to Western Galveston Bay were sampled in July 2023 in conjunction with the Environmental Institute of Houston (EIH).
- Water samples were acidified to capture phages and cells and concentrated via membrane filtration using duplicate 0.45-micron filters.
- DNA from each filter was extracted using commercial kit
- Next-gen sequencing was done on amplicons generated with primers for the V4 region.
- Sourcetracker2 used to estimate mixing of human waste
- Absolute quantification of crAssphages and *Enterococcus* species was performed using an Applied Biosystems QuantStudio™ in single 2-plex reaction

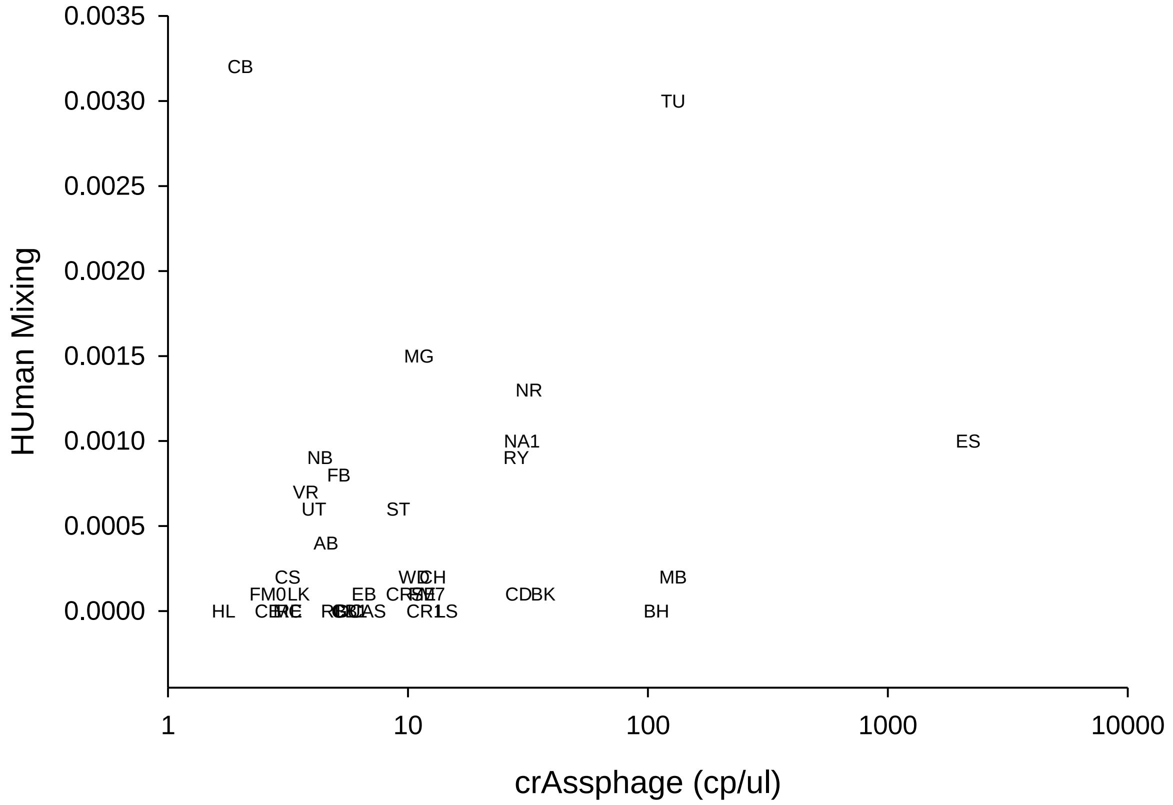
16s rRNA amplicon sequence variants

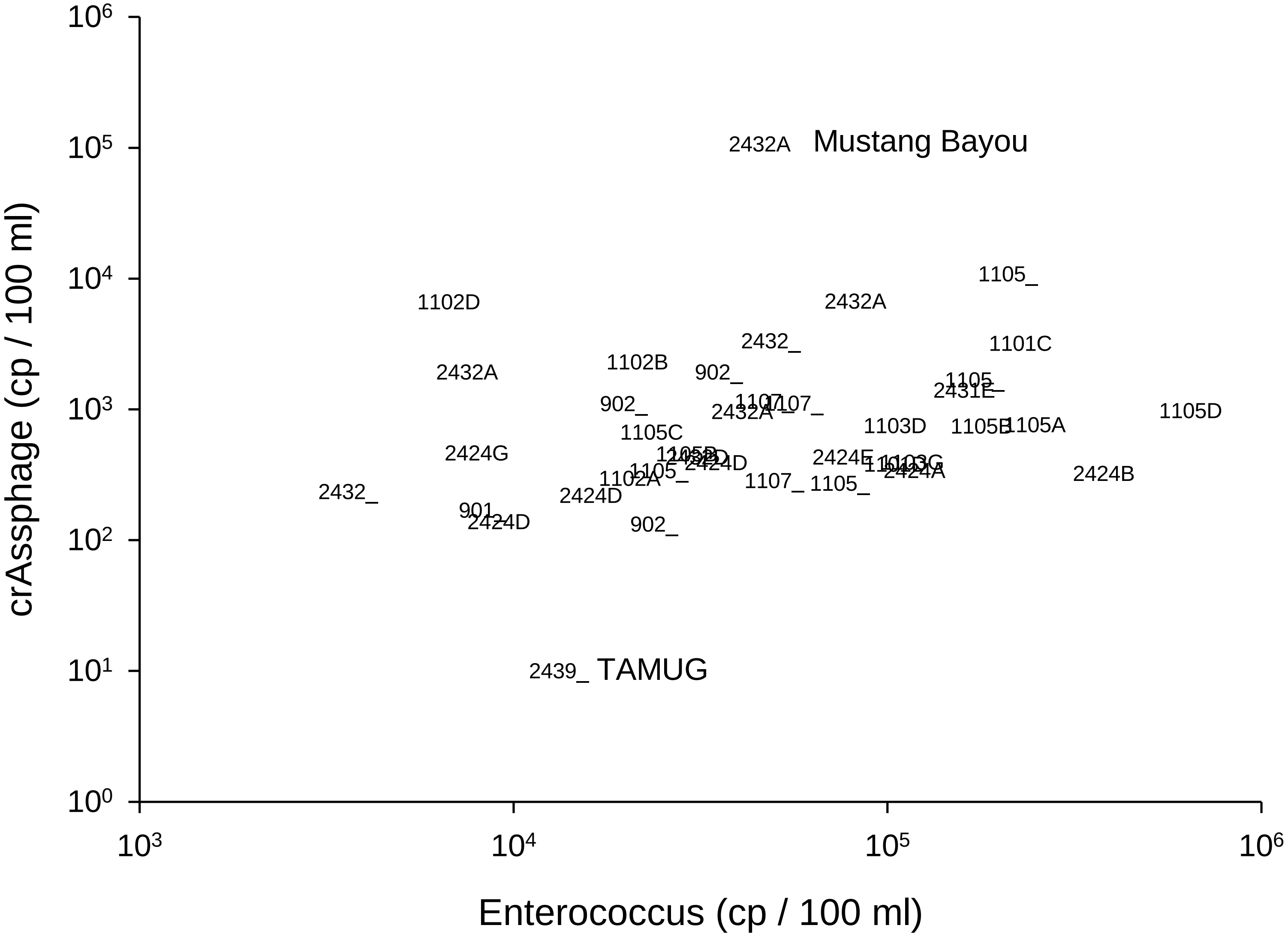


Non-metric multidimensional Scaling plot of bacterial community structure in tidal and non-tidal sites



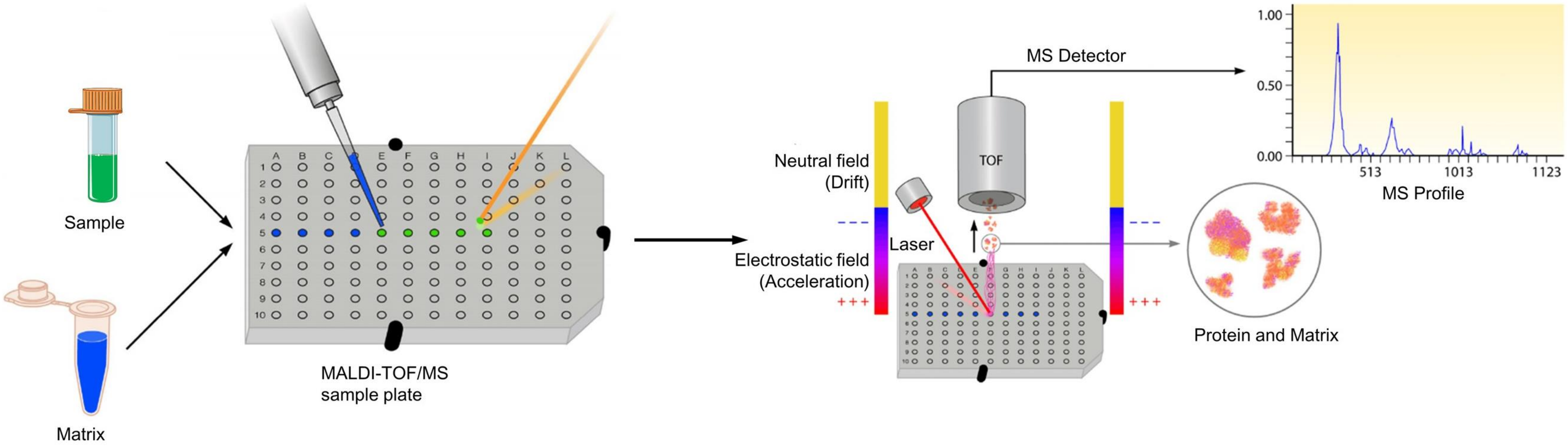
Weak Relationship between crAssphages and human source





crAssphage (cp / 100 ml)
10^6
10^5
10^4
10^3
10^2
10^1
10^0
2432A
Mustang Bayou
1105_
1102D
2432A
2432_
1101C
1102B
2432A
902_
1105_
2431E
902_
1107_
1107_
2432A
1105D
1103D
1105B
1105A
1105C
2424G
1105B
2424D
2424E
1103G
2424A
2424B
1105_
1102A
1107_
1105_
2432_
2424D
901_
2424D
902_
2439_
TAMUG
10^3
10^4
10^5
10^6
Enterococcus (cp / 100 ml)

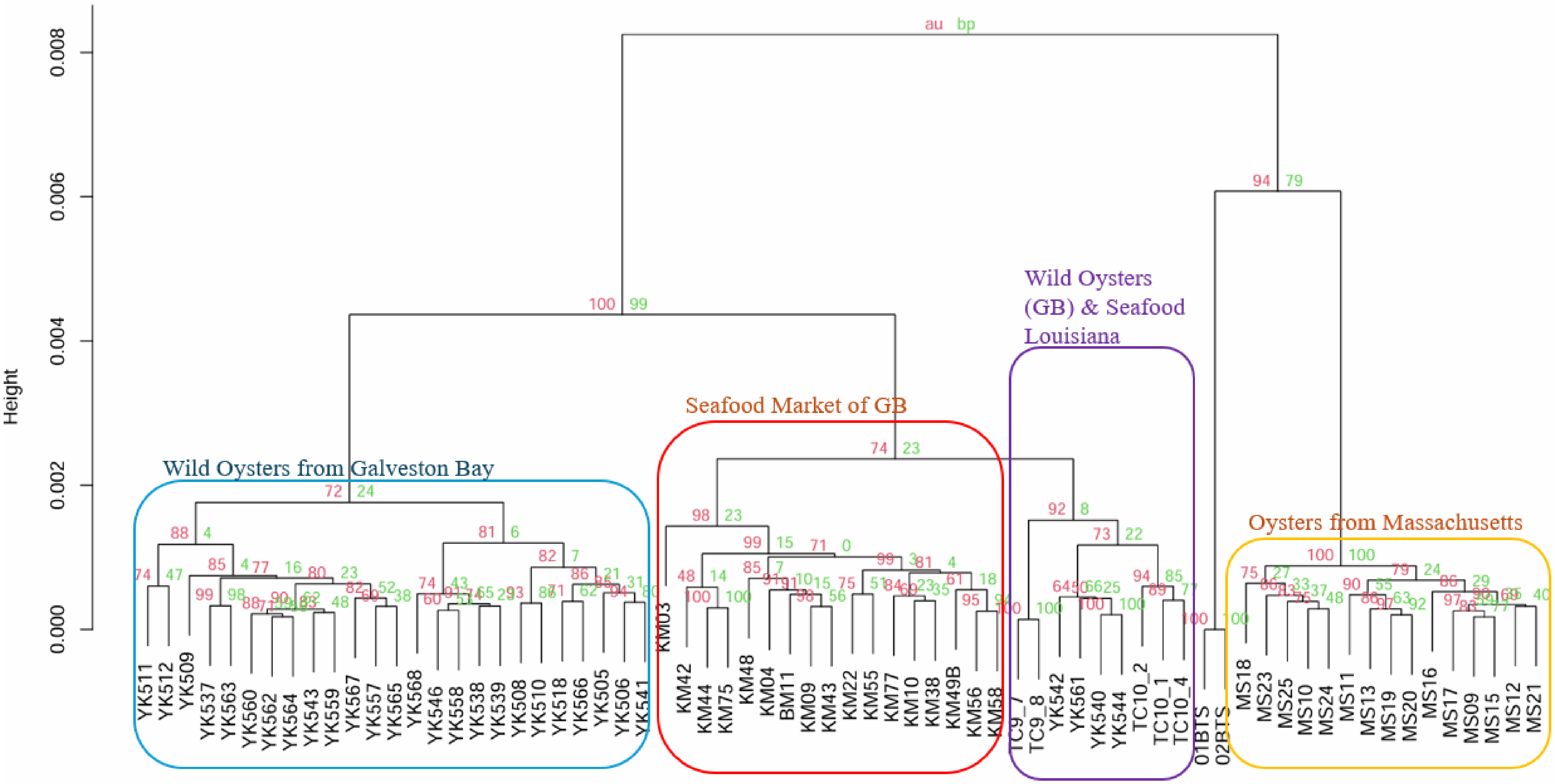
MALDI-TOF Mass Spectrometry



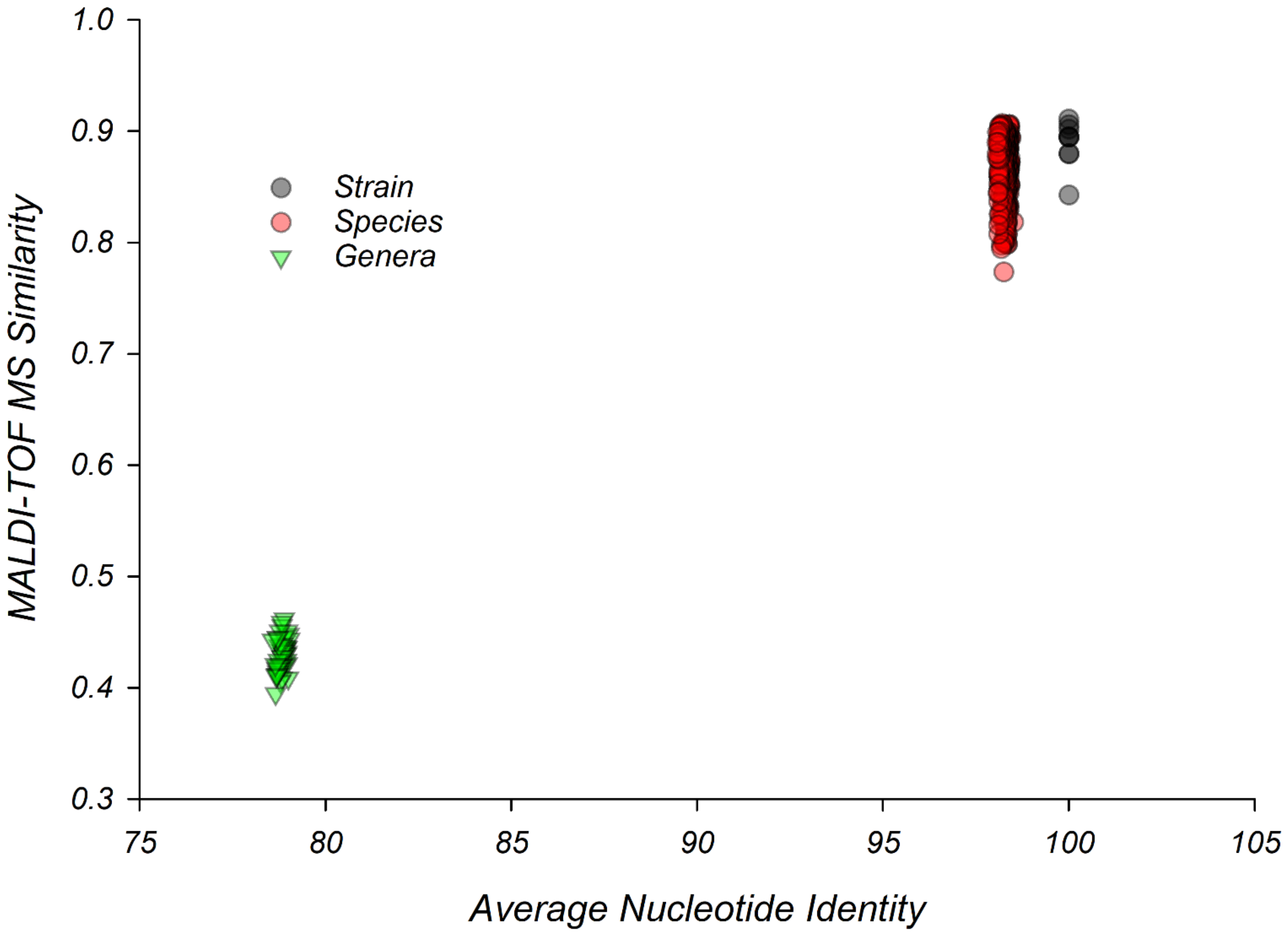
(Clark A. E., *et al.*; 2013)

- MALDI-TOF MS identifies isolates through protein characterization through molecular weight and peptide “fingerprinting”.
- Each isolates gets Bruker identification and score.
- Bruker scores above 2 are acceptable for species level identification; between 1.7-2 are acceptable for genus level identification; under 1.7 have no reliable ID.

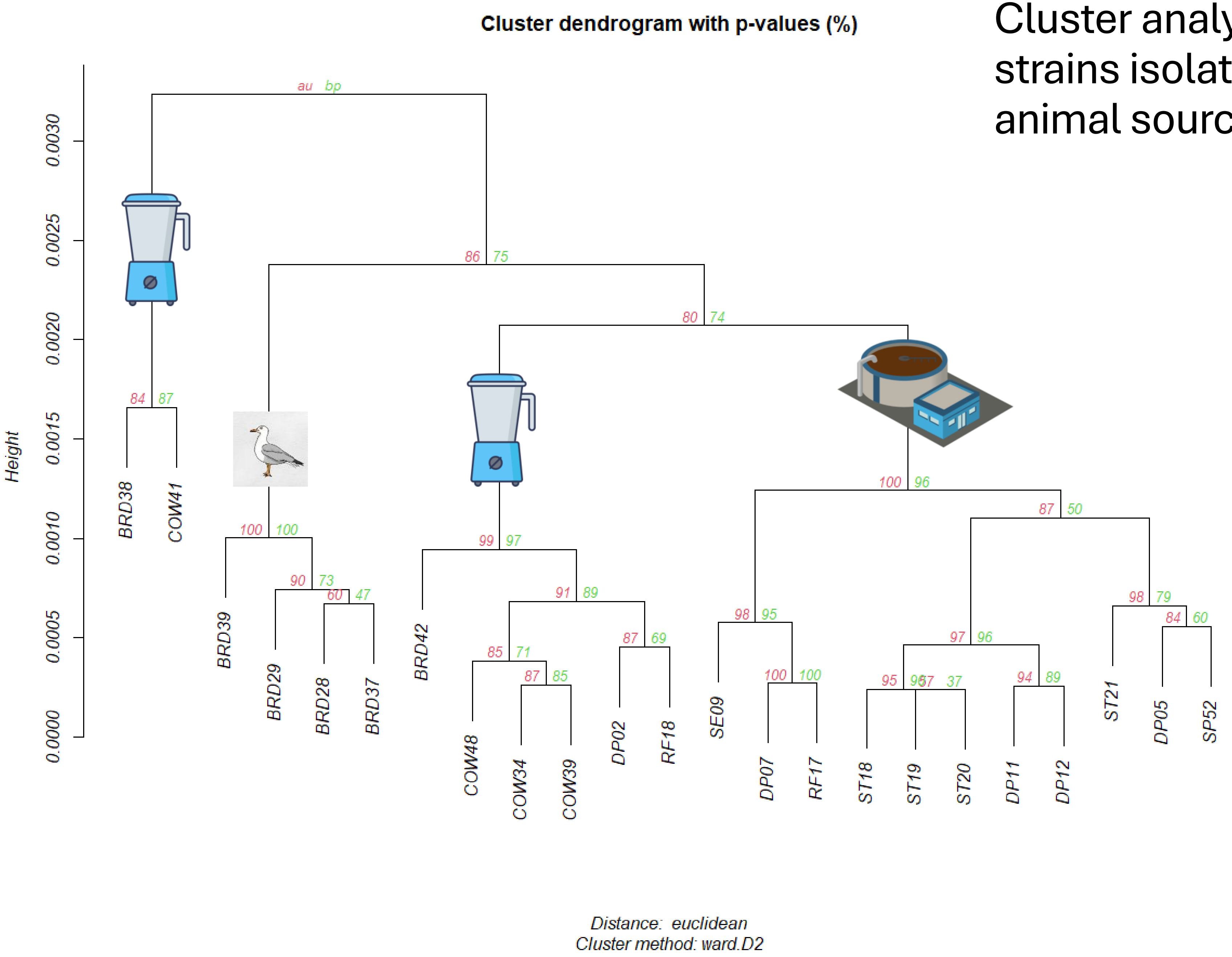
Cluster Dendrogram of *V.parahaemolyticus*



Comparison of Resolution of WGS to MALDI-TOF MS



Cluster analysis of *E. faecium* strains isolated from human and animal sources



- *Enterococcus* species isolated from various sources were identified with a MALDI Biotyper® system.
- Mass spectra generated from isolates that were identified as *E. faecium* selected for cluster analysis.
- Source-specific clusters associated with human waste or seagulls are indicated by icons.
- Clusters corresponding to a mixture of source types are indicated by the icon of a blender.

Conclusions

- dPCR and metagenomic analysis suggests fecal contamination from human sources is high in Mustang Bayou
- MALDI-TOF shows promise for MST

Acknowledgements

- This project received support from the EPA (02D18322) and NSF (2320765)
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  - dPCR
  - Targeted metagenomics
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- Tetyana King (*Vibrio)* and DSHS (WGS)