Shotgun metagenomic virulence factor detection for food safety screening of aquaponic culture water

Sean Fogarty¹, Todd Guerdat¹, Stephen Jones^{1,2}, Peter Konjoian¹

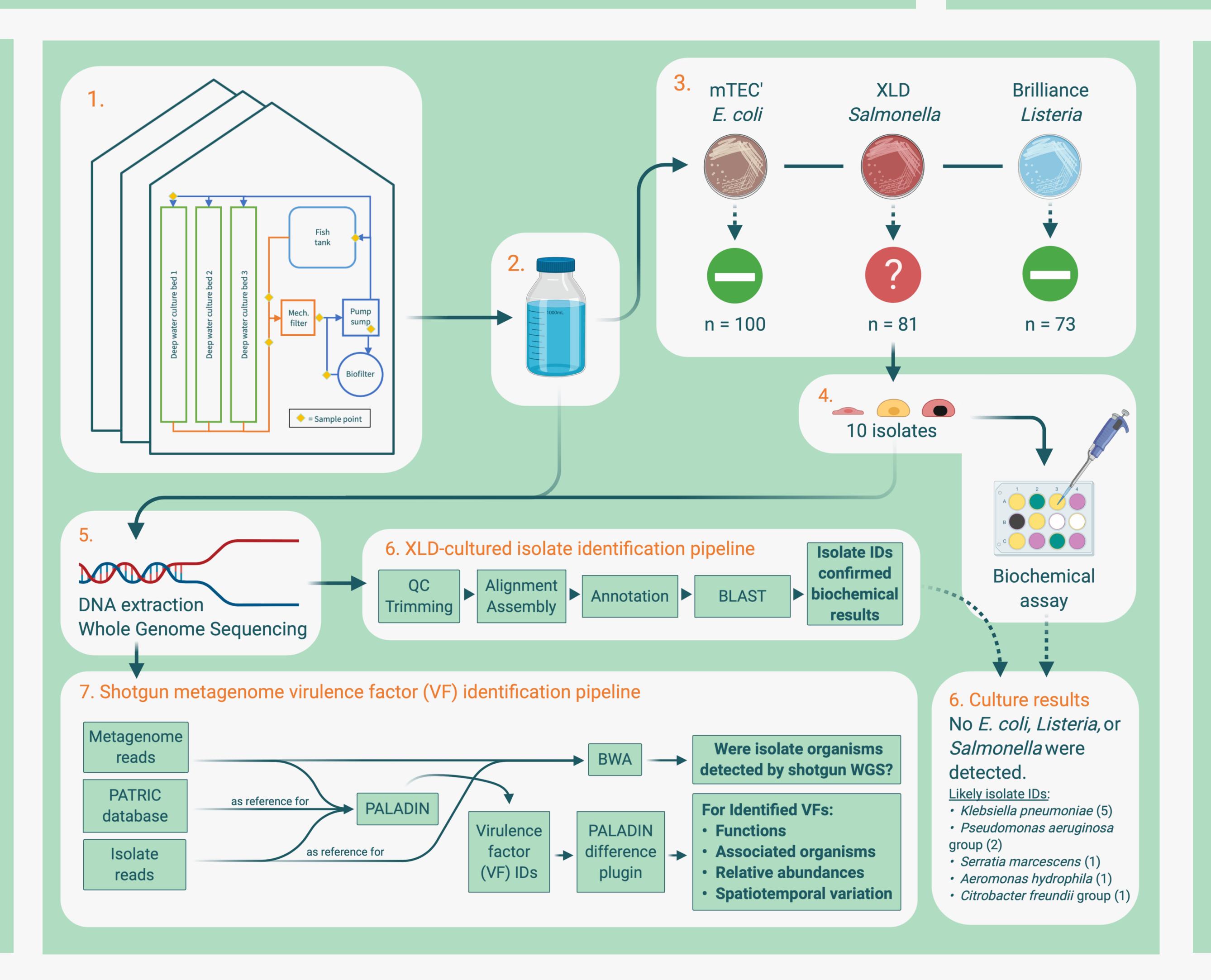
¹ University of New Hampshire, Durham, NH ² New Hampshire Sea Grant, Durham, NH Correspondence: <u>sean.z.fogarty@gmail.com</u>



University of New Hampshire NH Agricultural **Experiment Station**

Acknowledgements: Eternal thanks to Alex Sitek and the KFRAG crew for their tireless commitment to running a tight aquaponic ship. I could not have accomplished the lab work for this project without the help of my undergraduate assistants, Justine Cassel and Holly Hoag. Dhawal Darji, Lynette Davis, Gayathri Venkatasrinivasan, Toni Westbrook, and Kelley Thomas were instrumental in developing the bioinformatic pipeline.

- 1. Operated replicated aquaponic greenhouses for 1 year. We combined good agricultural practices (GAPs) established by industry with wastewater treatment principles to design a clean, healthy aquatic ecosystem. (See far right panel.)
- 2. Collected water samples at 8 sites in each system over an 8-week period.
- 3. Utilized an array of culture-based detection methods for human pathogens commonly carried on leafy greens.





United States Department of Agricultur National Institute of Food and Agriculture





Results & Discussion

- Culture-based methods (#3) did not detect 3 major human pathogens of concern in aquaponics.
- The conventional culture-based xyloselysine-deoxycholate (XLD) method for Salmonella detected many false positives and would not be suitable for screening culture water.
- However, biochemical (#4) and molecular (#6) confirmation of XLD isolates identified Gammaproteobacteria species closely related to many common pathogens, and some strains of these bacteria can be pathogenic.
 - 4. Biochemically disconfirmed presumptive positives for Salmonella spp. cultured on XLD.
 - 5. Extracted DNA from water samples and XLD isolates for whole genome sequencing (WGS).
 - 6. Utilized BLAST to confirm isolate identities through 16S rRNA sequence alignment.
 - 7. Currently developing a bioinformatic pipeline to analyze WGS isolate and metagenome data for known pathogen virulence factors (VFs).

- Our bioinformatic pipeline (#7) will detect pathogen virulence factors (VFs) in both aquatic metagenome and isolate samples. This will provide information about potential pathogen presence in the aquaponic metagenome, as well as possible pathogenicity of the XLD isolates.
- These forthcoming results will provide new insight into the food safety risk associated with aquaponic culture water. If favorable, they would be the first evidence that aquaponics may be used to safely and legally produce root vegetables that are typically eaten raw, such as radish.

Good agricultural practices (GAPs) for food safety in aquaponics

1. Worker and farm hygiene

- Worker training
- Biosecurity measures (e.g. footbaths, pest exclusion, hand washing)
- Regular facility cleaning
- Sanitization of tools and equipment
- Potable source water

2. System design and maintenance

- Aggressive solids removal
- Aeration in every unit process
- All water is treated before redistribution to fish and plants (see **#1** at left)
- Edible portion of crop does not contact culture water

3. System monitoring and control

- Daily water quality analysis for fish health management
- Operational checklists (AM and PM)
- Environmental monitoring and control systems provide real time alerts when out of range

References

Forstner, M. J. (2016). Salmonella Flipbook. Minnesota Department of Agriculture. https://www.fda.gov/files/food/published/%3Ci%3ESalmonella%3C-i%3E-Flipbook.pdf

Madigan, M. T., Bender, K. S., Buckley, D. H., Sattley, W. M., & Stahl, D. A. (2018). Brock biology of microorganisms (Fifteenth edition). Pearson.

Westbrook, A., Ramsdell, J., Schuelke, T., Normington, L., Bergeron, R. D., Thomas, W. K., & MacManes, M. D. (2017). PALADIN: Protein alignment for functional profiling whole metagenome shotgun data. *Bioinformatics*, *33*(10), 1473–1478. https://doi.org/10.1093/bioinformatics/btx021