Investigating the metabolic potential of *Salinivibrio* spp. as a potential industrial chassis

Crystal E. Young[1,2], Hussain Alattas[1,2], Harrison O'Sullivan[1,2], Daniel V. Murphy[1], & Colin Scott[1,3]

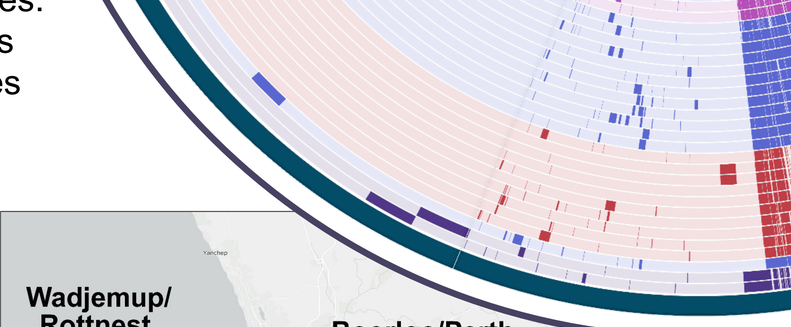
[1] Bioplastic Innovation Hub, Food Futures Institute, Murdoch University, Murdoch, Western Australia, Australia
[2] School of Medical, Molecular and Forensic Sciences, Murdoch University, Murdoch, Western Australia, Australia
[3] CSIRO Environment, Black Mountain Science and Innovation Park, Canberra, Australia

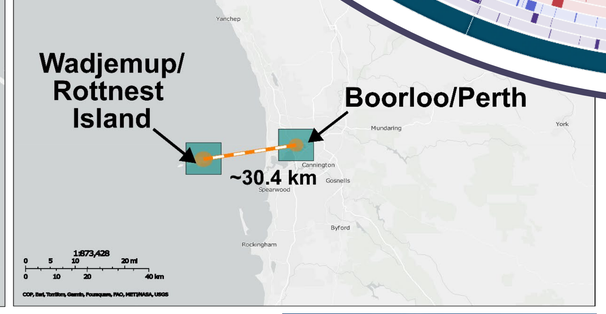
Introduction

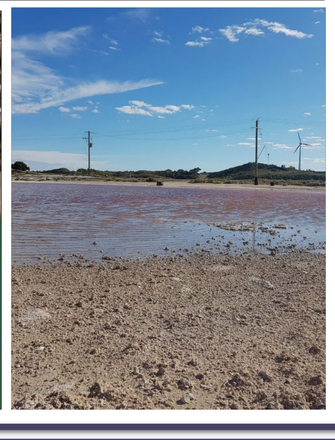
- Investigating hypersaline environments using culture-dependent methods offers an opportunity to isolate and store potential strains of importance for in-depth genomic analyses.
- Members of the genus *Salinivibrio* have recently gained interest due to their ability to produce valuable enzymes and biopolymers.
- *Salinivibrio* species are commonly isolated from hypersaline and saline environments because of their rapid growth on rich laboratory media.
- This rapid growth characteristic suggests that *Salinivibrio* could be an ideal chassis for bioplastic production where natural fast-growing microorganisms are often advantageous.

Isolation and phylogenetic analysis

- At the time of isolation, Pearse Lakes has a pH of 8.0±0.01 and salinity of 20.1±1.44%
- Whole genomes sequencing yielded nine completed genomes. ANI, dDDH, and MLSA analysis delineated the nine new isolates into their respective species.







PHA pathway analysis

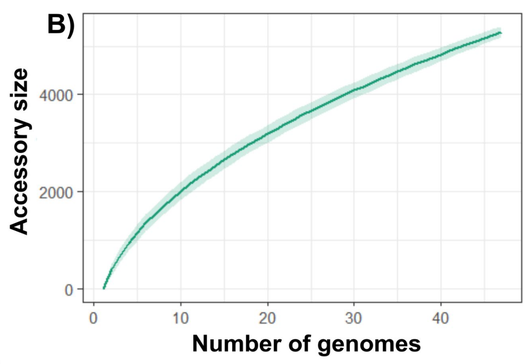
- Polyhydroxyalkanoate (PHA) production pathways were investigated in *Salinivibrio* strains, revealing that all analysed strains possess core pathway for poly(3-hydroxybutyrate) production.
- Initial analysis suggested the absence of PHA depolymerase (PhaZ) and PHA oligomer hydrolase (PhaY) genes, further investigation identified putative PhaZ proteins and other PHA catabolic enzymes

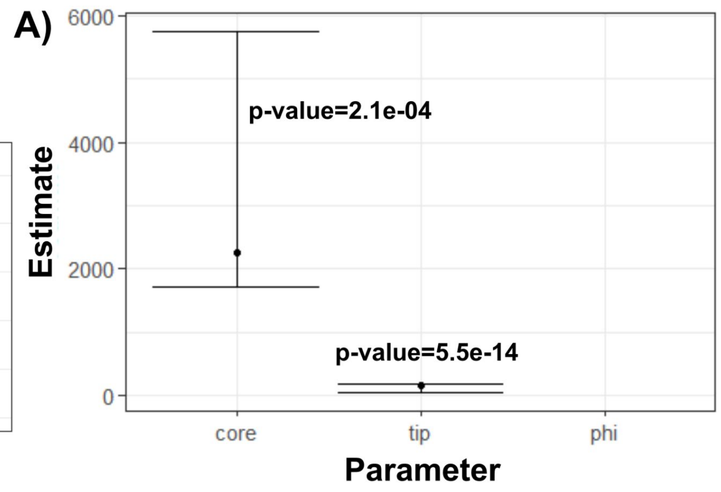
Methodology

1. Sample collection and isolation:• Isolated from Pearse Lakes, WA• Cultured in lysogeny broth (15% NaCl w/v) media
2. Genome sequencing and assembly:• Oxford nanopore sequencing• Flye assembly, quality checks (CheckM1 and BUSCO)
3. Comparative genomics and analysis:• ANI-BLAST and DDH for phylogeny• Anvi'o for pangenome analysis• COG and KEGG functional analysis

Pangenome analysis

- The *Salinivibrio* pangenome (47 genomes) was represented by 8,300 gene clusters (148,255 gene calls), of which the core genome contained 2,343 gene clusters (28%; 107,904 core genes) and accessory genome included 5,959 gene clusters (72%; 34,236 accessory genes).
- The model used to examine the rate of gene exchange events indicated a significant association between the core genome and branch length which indicates that the pangenome is 'open' indicating that *Salinivibrio* species may have a diverse accessory genome.





Metabolic pathway analysis

- Analysis of KEGG modules in 47 *Salinivibrio* genomes revealed 9,385 annotated modules, with 4,387 defined as completed, primarily in amino acid metabolism, cofactor and vitamin metabolism, carbohydrates, and energy metabolism.
- Central carbohydrate metabolism pathways were conserved in all genomes, while sulfate-sulfur assimilation and assimilatory sulfate reduction pathways were present in most strains;
- The study also identified completed pathways for isoprenoid and dTDP-L-rhamnose biosynthesis in several strains, potentially contributing to high salt adaptations.

Conclusion

1. *Salinivibrio* are genetically and metabolically well-suited for Industrial fermentation
2. We will adapt at least one strain for the production of bioplastics

www.linkedin.com/in/crystaleveyoung
https://orcid.org/0009-0001-3808-8672
C.young@murdoch.edu.au

Acknowledgements

This project was funded by a Murdoch University RTP scholarship (C.Y., H.O., & H.A) and a top-up scholarship through the CSIRO-Murdoch University-Industry Bioplastics Innovation Hub (C.Y., H.O., & H.A). H.A. was supported by a Murdoch International Postgraduate Scholarship. We acknowledge the Western Australia Department of Biodiversity, Conservation and Attractions for sampling permits. We acknowledge the Rottnest Island Authority for logistical assistance and access to Pearse Lakes. We also acknowledge the Whadjuk Noongar people as the original custodians of Wadjemup (Rottnest Island) and their continued connection to the island and its waters, and we pay our respects to elders past, present, and emerging.

References

Young, C. E., Alattas, H., Scott, C., Murphy, D. V., Tiwari, R., & Reeve, W. G. (2024). Complete genome sequence of a Marinococcus sp. PL1-022 isolated from the pink hypersaline Pearse Lakes, Rottnest Island, Western Australia. *Microbiology Resource Announcements*, e00129-00124.

Young, C. E., Alattas, H., Scott, C., Murphy, D. V., Tiwari, R., & Reeve, W. G. (2024). Complete genome sequence of Idiomarina sp. PL1-037 isolated from the pink hypersaline Pearse Lakes, Rottnest Island, Western Australia. *Microbiology Resource Announcements*, e00157-00124.