Monitoring Microbial Communities Diversity with Cytometric Fingerprinting

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Abstract Identifying and monitoring microbial diversity within microbial communities is critical to studying both natural and engineered biogeochemical processes. Most commonly, 16S rRNA amplicon sequencing is used to understand microbial community composition, but this process is labor and time intensive. Using FCM data of bacterial consortia from a produced water (PW) bioreactor, we intend to examine currently available R-based preprocessing and cytometric fingerprinting analysis packages to develop a pipeline best suited to monitor microbial communities.

Flow Cytometry

- Flow cytometry (FCM) is a process where information and phenotypic characteristics of individual cells are rapidly collected.
- The FCM targets individual cells with a laser, producing scattered light and fluorescence which reveal specific cell phenotypic characteristics.
- These cell signals can be preprocessed and analyzed with packages that can improve data visibility, remove noise, and analyze cell signals for cell population identification or cytometric fingerprinting.

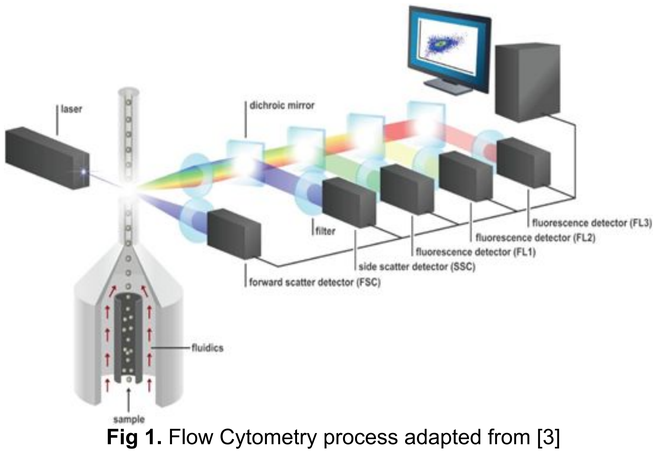

Fig 1. Flow Cytometry process adapted from [3]

Cytometric Fingerprinting

- Cytometric fingerprinting does not identify specific cells and instead focuses on identifying and visualizing the overall structure of a community and its subcommunities--providing visualization of heterogeneity within a community.
- This visualization allows for the monitoring of the entire community's structure and the changes in subcommunities
- Relies on phenotypic characteristics present in the community and the phenotypic characteristic distribution within the community

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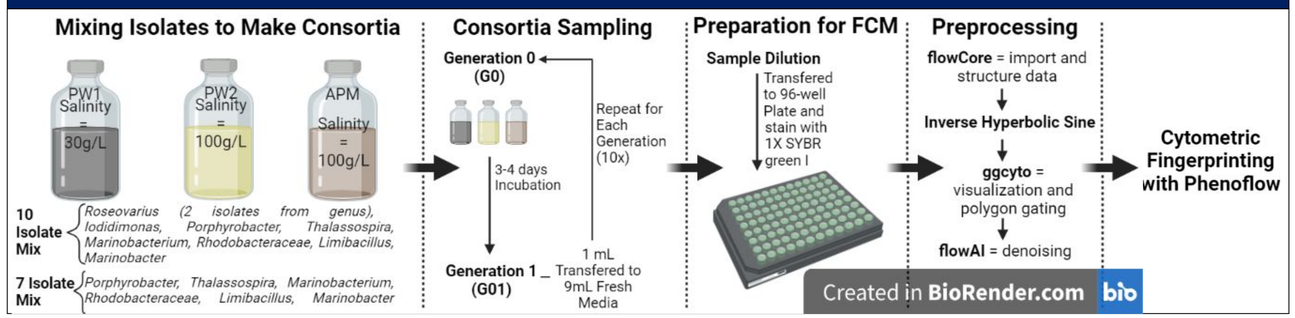
Materials and Methods



Diversity Index Plots

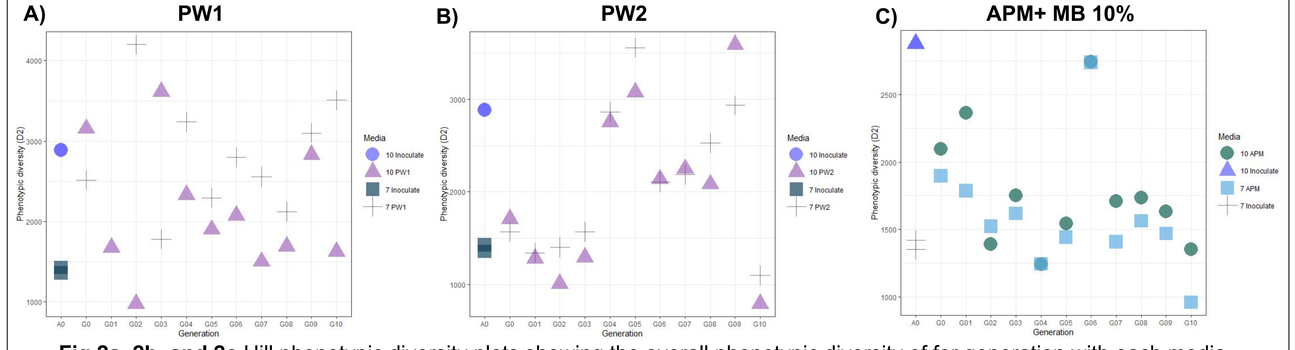


Fig 2a, 2b, and 2c Hill phenotypic diversity plots showing the overall phenotypic diversity of for generation with each media and consortia

NMDS of Media

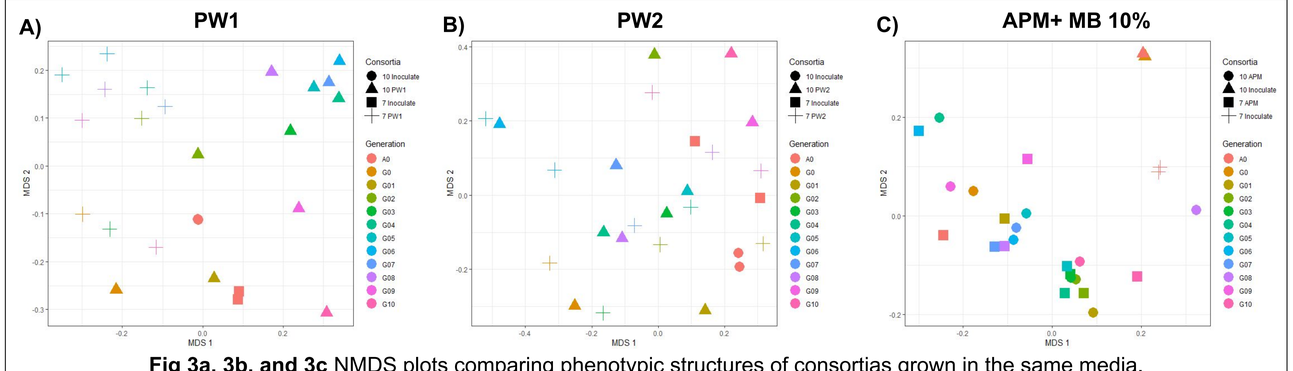


Fig 3a, 3b, and 3c NMDS plots comparing phenotypic structures of consortias grown in the same media.

NMDS of Consortia

7 Isolate Mix Consortia

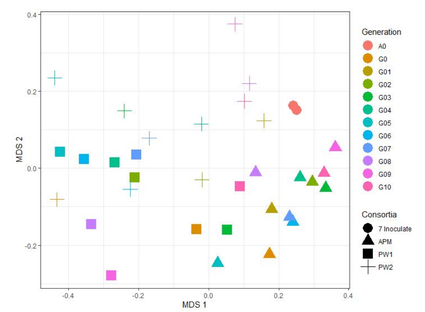


Fig 4 NMDS plot comparing phenotypic structures of 7 Isolate Mix Consortia with different media.

10 Isolate Mix Consortia

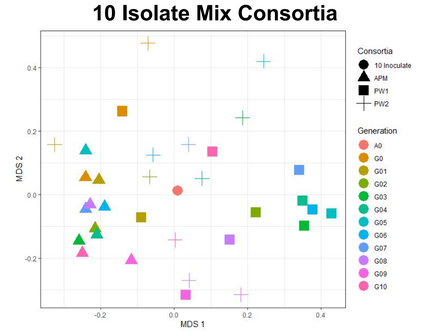


Fig 5 NMDS plot comparing phenotypic structures of 10 Isolate Mix Consortia with different media.

Conclusion

- Cytometric fingerprinting workflow selected allows monitoring of overall cell count, phenotypic diversity, and the phenotypic similarities between generations.
- Phenotypic structure of communities are strongly dependent on the media; APM may select for few fast-growing species; PW1 and PW2 media may support greater number of species.
- Additional validation using 16s rRNA sequencing is necessary.

Acknowledgements

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