Advancing flow cytometry in the water sector

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Introduction and background

- Microbiological drinking water quality (MDWQ) is currently assessed using culture plates, which rely on growth of bacteria (requiring 16 to 72 hours depending on method). As a result, data on MDWQ is only available after the water has been supplied.
- Analysis by flow cytometry can provide a rapid assessment of MDWQ. A protocol developed in Switzerland [1] is currently being applied by many UK water providers to measure total and intact cell counts in drinking water samples in ~20minutes of sample processing and analysis.
- Flow cytometry (FC) gives multiparametric data on individual cells, and specific stains can be used to identify similar populations of cells in heterogenous populations, a feature which is underutilized in the current protocol.
- DNA binding stain SYBR Green I has been used to separate cells based on DNA content into high nucleic acid (HNA) and low nucleic acid (LNA).
- There is some evidence that HNA and LNA groups in environmental samples are associated with different phyla of bacteria [2].

Initial experiments

- To study the relationship between genome size and HNA and LNA, bacteria of known genomes sizes were grown.
- Cultures were stained with SYBR Green I over the course of their growth cycle.
- Green fluorescence emission was measured at points corresponding with lag, exponential and stationary phase.

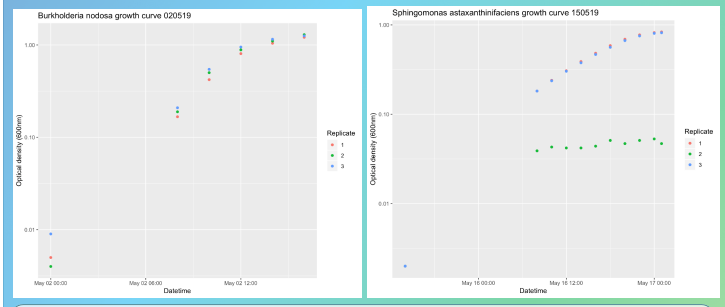

Figure 1. Growth curves of *Burkholderia nodosa* (left) and *Sphingomonas astaxanthinifaciens* (right) measured at optical density (OD) 600nm.

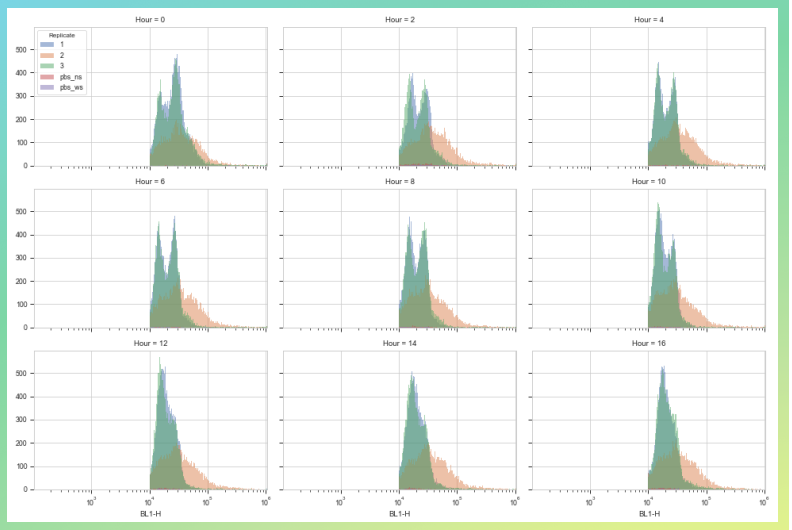

Figure 3. Green fluorescence intensity of *Sphingomonas astaxanthinifaciens* (genome size = 2.5Mb) over growth. Note: replicate 2 did not grow.

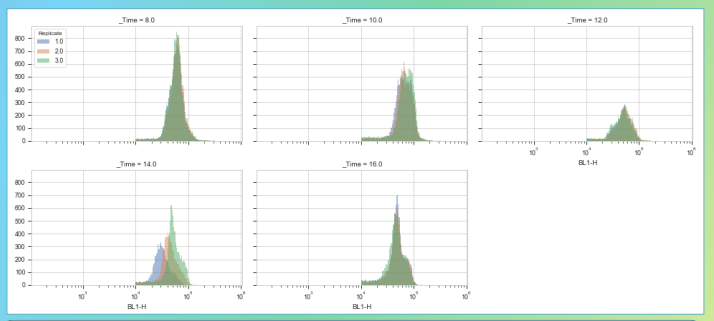

Figure 2. Green fluorescence intensity of *Burkholderia nodosa* (genome size = 9.6Mb) over growth.

Conclusions and further work

- Small genome bacteria (~LNA) may overlap in green fluorescence emission with large genome bacteria (~HNA) during replication, due to increased DNA within cells.
- *B. nodosa* do not produce a dual peak of green fluorescence during growth phase. This could be due to different cellular DNA replication and division systems in the two bacteria.
- Other bacteria of different known genome sizes will undergo fluorescence analysis over growth to see how this effects their classification as HNA or LNA, these are: *Azohydromonas australica* (8.7Mb), *Runella zeae* (7.6Mb), *Lactobacillus psittaci* (1.5Mb), *Wohlfahrtiimonas chitiniclastica* (1.9Mb).
- A portfolio of different DNA specific dyes will be used in combination to create further divisions of bacterial populations based on DNA-linked fluorescence emission, e.g. AT-linked fluorescence vs total DNA fluorescence

Results

- Large genome bacteria *B. nodosa* (Fig. 2) have increased green fluorescence emission per single cell compared to *S. astaxanthinifaciens* (Fig. 3).
- A dual peak of green fluorescence emission can be seen from *S. astaxanthinifaciens* (Fig. 3) at points corresponding to log growth, as measured by OD600nm (Fig. 1 right).

References

[1] SLMB . "Determining the Total Cell Count and Ratios of High and Low Nucleic Acid Content Cells in Freshwater Using Flow Cytometry." Analysis Method 333.1, the Swiss Food Book (Schweizerische Lebensmittelbuch) (2012)

[2] Proctor, Caitlin R., et al. "Phylogenetic clustering of small low nucleic acid-content bacteria across diverse freshwater ecosystems." *The ISME journal* 12.5 (2018): 1344.

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