The Effect of Starvation and Drift on Microbial Traits Across Phylogenies

The purpose of this document is to outline what trait-associated data will be collected during the course of MURI2 task 2

We can use population genomic data to look at the genetic basis behind these traits

We have a fairly good outline on what sort of "-omic" data will be collected throughout task 2, however little discussion has been had over what trait-associated data to collect.

Viability counts

This will be done through basic plate counts on MURI media agar.

Live/dead counts with respiration-associated fluorescence.

Pat Foster's new technician is taking over the job of microscopy from Ellen for task 1.

It would be nice to have assistance during sampling days due to how large and time consuming transferring can be.

However, we have the optic cube on our Nikon scope that allows us assess respiration using CTC. This would be useful data to have along with live/ dead cell counts.

Flow cytometry

An issue with microbes is how do you collect quantitative trait data of individual cells.

We can use this data to look at how respiratory activity changes over time, informing us about the formation of a seed bank. Preliminary data from *Janthinobacterium* suggests this, but I will be generating more preliminary data from the flow center here.

Every six months we can look at the total number of respiring cells, dead cells, and viable cells using flow cytometry. This will have to be done on the actual culture and will (hopefully) only require about 100 uL.

Maximum specific growth rate & lag time

This can be calculated for each strain using the plate reader. We can only run 48 samples at a time (including controls), so we will have to use frozen stocks if we want to assess maximum

growth rate and time in lag phase at the same timepoint for all lines in the experiment.

We could plate out frozen stocks, pick 3 colonies, run three replicates of each colony, allowing for us to assess the maximum growth rate of five lines at a time. I'm envisioning getting an undergrad to help with this.

Biofilm formation

For this we can just run an O'Toole assay using the plate reader. Again, I'm hoping to get an undergrad to help with this.

Motility

Follow the protocol in Lennon et al., 2012. Spot 5 mL of log phase culture on the middle of a semi-solid agar plate (1% agarose?). Simple, cheap, I can easily work with an undergraduate.

Here we will have to grow up a colony from the frozen stocks generated throughout the experiment.

We may want to do additional tests throughout the experiment, but I see this as a good place to start.

Comments/ questions/ concerns?