7: Invasion of L Plantarum LF into L. Plantarum HS

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**Overview and goals:**

The goal of this experiment is to determine how different fitness rates determine species composition. Hopefully we would find that the results match our voltage equation. At the end of this experiment, we should know:

* How fitness differences affect final community composition.
* How to perform experiments like this with flow cytometer
* How to use the robot to perform this type of experiment

We performed a preliminary experiment similar to the growth curve experiment from experiments 1 and 3 to determine what strain to use. Based on the OD600, fluorescence data and the confounding variable of Dam HI endogenous CAM resistance, I decided that I will be using LP\_HS\_GFP as the indigenous species, and LP\_LF\_mCherry as the invader.

**Two days before Experiment(5/10):**

* Grow 3 tubes of LP\_HS\_GFP and LP\_LF\_mCherry from 3 separate colonies from plates in 3 mL MSR+CAM. Grow a tube of LP\_WT as a control.

**One day before the experiment (5/11):**

* Back dilute each tube 1/1000 LP\_HS\_GFP and LP\_LF\_mCherry from 3 separate colonies from plates in 9 mL MSR+CAM.

**Day 1 (5/12):**

* Back dilute each culture to an OD of 0.1. Grow up to about an OD of 0.7-1. We want many mL of indigenous culture and about 1 tube of 6 mL culture for each dilution of invader.
* Spin down invader and resuspend in PBS (2x)
* Setup plate. Vertically will be dilutions of invader, horizontally will be replicates of indigenous bacteria. 1 mL of each indigenous culture in each well of the deep well. Add 500uL of 2x MRS, 500uL of 0.25,0.5,1, 2x invader. Remove 1 mL.
* Dilute every 1.5 hrs with 500uL of 2x MRS and 500 uL invader, sample every 2uL 30 minutes onto 198 uL of PBS. Repeat for 6 cycles (9 hours).

**Day 1 Onward (5/12):**

* Obtain cell counts with flow cytometer use 10uL at 25 uL/sec. Use Ren’s experimental settings.

**Robot Plan**

Plates required:

6 sample plates

4 condition plates

Deep well Experimental plate

MRS vat

P1000 tips

P20 tips

**Procedure**:

Add 500 uL invader culture in PBS. Premix 3 times in condition plate, then mix in sample plate 3 times with

Add 500uL MRS. mix 3 times. Then remove 1000 uL mixed culture.

Remove 2uL and add to sample plate (first 4 wells)

Wait 30 minutes

Mix with 500 uL 3 times

Remove 2 uL and add to sample plate (second 4 wells)

Wait 30 minutes

Mix with 500 uL 3 times

Remove 2 uL and add to sample plate (third 4 wells)