4: Survival of L.Plantarum in PBS

*Josh Derrick 2021-04*

**Overview and goals:**

The goal of this experiment is to determine how many LP\_HS survive in PBS over the course of a time course. This would be useful for us because if we can keep log-phase bacteria of one strain at a constant OD, it will make it easier to pulse in bacteria rather than having to constantly dilute them to keep them at the same OD

At the end of this experiment, we should:

* Know the Survival rate of L plantarum on PBS.
* Know what the growth rate of L Plantarum on PBS is after recovery in media

**Three days before Experiment(4/11):**

* Streak LP\_HS\_GFP and LP\_HS\_mCherry from Glycerol on plates of MSR+CAM. Grow up to 24 hours. Also streak LP\_WT as a control
* Prepare MSR+CAM
* Prepare PBS+CAM

**One day before the experiment (4/13):**

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

**Day 1 (4/14):**

* Dilute LP replicates in MSR+CAM to an OD of 0.5. Read the OD using the cuvette reader in lab
* Further dilute this OD of 0.5 culture to an OD of 0.05. Grow until the culture reaches log-phase around an OD of 0.25-0.5. Check OD every 30 minutes.
* Pellet with centrifuge and resuspend in PBS.
* Plate ~50 uL on CAM plate (maybe dilute)
* Setup plate for plate reader with 200uL. Take readings overnight. Use a temperature gradient from 4 C to 25 C.
* Leave at RT with shaking overnight.
* Keep an aloquot (1 mL) in 4 C

**Day 3 (4/15):**

* Plate 50 uL from the culture in the morning as well as from plate reader (do dilution series)
* Grow culture from PBS in new media in plate reader.

**Day 4 Onwards (4/16)**

* Count colonies and find dropout rate over time.
* Find growth rate of recovered colonies