Persister Time Course Killing Assay

Purpose: To determine the percentage of drug persisters formed in a given bacterial population after treatment with antibiotics, a bacteriostatic drug, or both. The time course killing assay is also used to determine the mono or biphasic killing curve of the bacterial population after the addition of an antibiotic or drug**. Note: This assay is time sensitive and necessary dilutions, reagents, and plates should be prepared in advance.**

Materials:

- LB plates
- 1x PBS
- Antibiotic Stock
- Drug Stock
- Overnight or exponential phase bacterial cultures
- 1.5 mL microcentrifuge tubes
- Sterile loops

Procedure:

1. Grow a single colony from each of your strains overnight (~15-18 hrs) in 3 mL LB media with shaking (200 rpm) at 37°C.
2. Subculture 10 µL of your overnight cultures into 10 mL (1:1000 dilution) of fresh prewarmed LB media. Grow at 37°C with shaking (200 rpm) for 2-3 hours, or until the OD reaches 0.2. *While your subcultures are growing, prepare your 1x PBS dilution tubes for your first sample.*
3. Once the OD reaches 0.2, add 100 µL of each culture to 900 µL of 1x PBS. This sample will be your t=0. *PBS is an ATP deficient media where cultures will not be able to grow. However, cells can still divide unevenly and should not be left sitting in the PBS for extended periods of time.*
4. **Immediately** add 10x the MIC of your antibiotic to your 10 mL of culture. For ampicillin, this would be 8 µL of 100 mg/mL ampicillin for a final concentration of 80 µg/mL. Allow to grow for an additional hour before taking your next sample.
5. Depending on the density of your t=0 culture, you may have to further dilute your t=0 sample in PBS by 10 or 100-fold. The goal dilution to plate is 10^5x from your original culture.
6. Divide an LB plate into 4 wedges on the agar side. Spot 10 µL of your diluted sample onto the wedge, then spread evenly within the wedge using a sterile loop.
7. Every 1-2 hours after adding the drug, remove 1.0 mL of culture from each strain, and spin it down at 4,200 x g (rcf) for 3 minutes in a 1.5 mL microcentrifuge tube.
8. Pour off the supernatant and wash once in 1x PBS. *To wash cells, resuspend them in 1.0 mL 1x PBS, pellet them as before, pour off the supernatant, tap off residual media on a Kimwipe, and repeat.*
9. Resuspend the washed cells in 1.0 mL fresh 1x PBS (1x dilute). If the washed pellet was large, further dilute the washed cells 10-fold, then plate both the 1x and 10x dilutions on your LB spot plate.
10. Repeat the process every hour for 6-8 hours after t=0.

11. Allow plates to grow overnight (20-24 hrs) agar side up in the 37°C incubator.
12. The following day, count the number of colonies that grew in each wedge. These will be the persister cells that survived the drug treatment. Average the number of colonies between each sample accordingly. Multiply the average number of colonies by the dilution factor plated to get the resulting CFU/mL at a given time point.

Example:

Average Colonies	14 colonies
Dilution Factor	10^3 (PBS dilution) x 10 (µL plated)
Total CFU/mL at t=X	1.4 x 10^4 CFU/mL