Force Evolution of L.rhamnosus and B.subtilis

Introduction

After following the protocol for making lead plates, this is WPI iGEMs protocol for force evolving L.rhamnosus and B.subtilis to uptake more lead. We want these bacterial strains to uptake more lead so that either of these GRAS organisms could be used as a prophylactic probiotic. This protocol explains two differing routes of accomplishing this goal. One route force evolution by streaking, which can be quantified by the WPI iGEMs Lead Assay telling us the amount of lead the bacteria is binding. The other route is force evolution through serial dilutions which can be quantified by counting colonies and studying the inhibition of growth when exposed to varying lead concentrations.

Materials

- Freshly streaked L.rhamnosus (on control MRS plate)
- Freshly streaked B.subtilis (on control LB plate)
- Varying concentration of lead plates
- LB Broth (autoclaved)
- MRS Broth (autoclaved)
- Micropippetors
- Automative Pipetteman
- Serological Pipettes
- 15 mL conical tubes
- Spectrophotometer
- Clicker counter
- Incubator set to 37 degrees Celsius

Procedure

Force Evolution by Streaking

1. Make sure you have a freshly streaked plates of your bacteria if not, streak a new plate and leave in the incubator for 24 hours to grow overnight
2. Pick one individual colony from the freshly streaked control plate and streak onto a lead plate of your lowest concentration

   Repeat on the same concentration (should have 2 plates to compare for every concentration)
3. Pick another colony and streak onto a higher lead concentration
4. Pick another colony and streak onto a new control plate
5. Place all your plates in the incubator to grow overnight

6. Check them at the 24 hour point, and if individual colonies have formed, remove from incubator

7. If not (L.rhamnosus takes 48 hours), leave for another 24 hours.

8. Look at these colonies under a microscope, and see if there is another change in morphology from control -> highest lead concentration and record in your notebook.

9. Using WPI iGEM Lead Assay Protoccol, edited from the Hach LeadTrac Kit the amount of lead this bacteria bound can be quantified

10. Next, streaking the colonies from the lead plates onto higher lead plates and seeing if they can survive/grow and then measure the binding again seeing if there was an increase in lead binding capabilities

Force Evolution by Serial Dilutions

11. First step, is finding a serial dilution that gives you within 100-200 colonies of your bacteria

    1. Make an overnight culture (10mL) of your bacteria within its media of choice and grow for 14-18 hours
    2. Measure OD600 in a spectrophotometer of an undiluted sample
    3. Now create serial dilutions all the way up to 1,000,000 (total of 6 dilutions)
    Quick reminder of Serial Dilution Protocol: Set up in 15 mL conical tubes, using a serological pipette with a pipette man place 9mL of sterile media broth inside a conical tube. Take 1mL for the undiluted overnight culture and place in 9mL sterile media within the tube. Mix by inverting the tube multiple times as well as pipetting up and down, this is 1:10 serial dilution. Then take 1mL from the 1:10 serial dilution, and place into a new 15mL conical with 9mL of fresh sterile media.
    4. 100 uL of the serial dilution was plated and spread on control plates (no lead). This was done twice for every concentration (2 plates per dilution)
    5. Place in incubator overnight - 48 hours and colony count to determine the perfect dilution to move forward with.

12. Once a serial dilution is picked (our perfect dilution was in between 1:100,000 and 1:1,000,000), one can move forward with plating this serial dilution onto lead plates.

13. Create an overnight culture once again, and performing serial dilutions to your desired dilution the next morning

14. Plate 100uL of the serial dilution onto varying lead concentration plates with a control using glass beads (2 plates per dilution and concentration).

15. Place all plates into the 37 degree incubator

16. After the growth period overnight -48 hours , removing the plates and counting the individual colonies using a clicker counter

    Record each amount

17. After collecting all the data, anaylzing the data in excel by graphing and using trend lines to see if an inhibition of growth was seen with varying lead concentrations.

18. This colonies can also be restreaked onto higher lead plates and tested with WPI iGEMs Lead Assay