6-14-19 Initial practice for growth protocol

Thursday, June 6, 2019 10:27 AM

Start with 3 Pseudomonas isolates
1 biological replicates
2 technical replicates

Day 1:

- [ ] 1. Streak isolates onto BHI agar plate
- [ ] 2. Incubate at 32°C overnight (16-18 h)

Day 2:

- [ ] 1. Inoculate 5 mL BHI broth with a single colony
- [ ] 2. Vortex for 5 seconds
- [ ] 3. Incubate tube at 32°C overnight (16-18h)

Day 3:

- [ ] 1. Vortex culture tube for 5 seconds
- [ ] 2. Perform serial dilutions in PBS (transferring 100 uL into 900 uL PBS) to the 10^{-5} dilution
- [ ] 3. Spiral plate 50 uL of sample in duplicate onto BHI agar plates and incubate at 32°C overnight (16-18h)
- [ ] 4. Store inoculated BHI tubes at 4°C overnight (these will be used to inoculate milk on day 4).

Day 4:

- [ ] 1. Enumerate cfu on BHI plates using Q count from your overnight culture and record in excel spreadsheet.
  - Past data showed that bacterial numbers ranged between 7.0×10^7 and 1.3×10^8 cfu/mL
- [ ] 2. Adjust with PBS to achieve an inoculum concentration of 100 cfu/mL
- [ ] 3. Prepare inoculums: resuspend the desired culture in media (SMB milk). Transfer 1 mL of resuspended culture to 9 mL of uninoculated media (SMB milk), for a total of 3 tubes per media tested. Vortex for 5 seconds to resuspend.
- [ ] 4. Remove 500 uL for plating and, using the spiral plater, spiral plate both an undiluted culture (from step 2) and the 10^{-1} dilution (from step 4) onto BHI plates in duplicate.
- [ ] 5. Incubate inoculated milk tubes at 6°C, and the spiral-plated agar plates at 32°C overnight (16-18 h). Be sure to include 1 uninoculated control tube which will be used to account for potential contaminants. (8AM)

- [ ] 6. Remove tubes from the 6°C incubator and vortex for 5 seconds. (8PM)
- [ ] 7. Aseptically transfer 500 uL of cultures into 1.5 mL Eppendorf tubes. Return tubes to 6°C incubator.
- [ ] 8. Perform serial dilutions to 10^{-5}.
- [ ] 9. Plate undiluted, 10^{-2}, and 10^{-4} dilutions
- [ ] 10. Incubate inoculated plates at 32°C for 24 hrs.

Day 5-6

- [ ] 11. Enumerate starting inoculum using the Q-count, to ensure the inoculum was 100 cfu/mL. Record results in excel spreadsheet.
- [ ] 1. If the inoculum was between 90 – 200 cfu/mL the tubes will continue to be incubated, if not, repeat days 2-5.

- [ ] 2. Remove tubes from the 6°C incubator and vortex for 5 seconds. (8AM)
- [ ] 3. Aseptically transfer 500 uL of cultures into 1.5 mL Eppendorf tubes. Return tubes to 6°C incubator.
- [ ] 4. Perform serial dilutions to 10^{-5}.
- [ ] 5. Plate undiluted, 10^{-2}, and 10^{-4} dilutions
- [ ] 6. Incubate inoculated plates at 32°C for 24 hrs.

- [ ] 7. Remove tubes from the 6°C incubator and vortex for 5 seconds. (8PM)
- [ ] 8. Aseptically transfer 500 uL of cultures into 1.5 mL Eppendorf tubes. Return tubes to 6°C incubator.
- [ ] 9. Perform serial dilutions to 10^{-5}.
- [ ] 10. Plate undiluted, 10^{-2}, and 10^{-4} dilutions
- [ ] 11. Incubate inoculated plates at 32°C for 24 hrs.

Day 7

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- [ ] 1. Enumerate inoculum using the Q-count. Record results in excel spreadsheet.
- [ ] 2. Remove tubes from the 6°C incubator and vortex for 5 seconds. (8AM)
- [ ] 3. Aseptically transfer 500 uL of cultures into 1.5 mL Eppendorf tubes. Return tubes to 6°C incubator.
- [ ] 4. Perform serial dilutions to 10^{-5}.
- [ ] 5. Plate undiluted, 10^{-2}, and 10^{-4} dilutions
- [ ] 6. Incubate inoculated plates at 32°C for 24 hrs.

Day 8

- [ ] 7. Repeat day 7 until Nmax is reached
    - [ ] • When the count doesn't increase in two platings in a row
- [ ] 1. Calculate the growth rate by subtracting the initial-day concentration from the final-day concentration
- [ ] 2. Build growth curve