**Secret’s in the Milk: Testing Standard Milk Media for Supporting Growth of Psychrotolerant Bacteria**

Objectives:

1. Summarize current literature on growth of psychrotolerant bacteria in milk
2. Quantify differences in growth rate for Gram-negative and Gram-positive psychrotolerant bacteria in different milk medias
3. Determine which media should be used for quantifying growth capability at refrigerated temperatures

**Experimental Outline:**

You will test *Paenibacillus* spp., *Bacillus cereus* group, and *Pseudomonas* spp. isolates in 3 different ‘milk medias.’ Growth will be assessed at 6°C at 0, 14, and 21 days.

|  |  |  |
| --- | --- | --- |
| Genus | Isolate ID | Location |
| *Paenibacillus* | F4-0126 | F1 Tower 104 |
| *Paenibacillus* | H7-0918 | F3 Tower 310 |
| *Paenibacillus* | F4-0202 | F1 Tower 104 |
| *Pseudomonas* | R10-2149 |  |
| *Pseudomonas* | R10-2286 |  |
| *Pseudomonas* | R10-2671 |  |
| *Bacillus* | E2-0214 | F3 Tower 325 |
| *Bacillus* | M7-0669 | F3 Tower 323 |
| *Bacillus* | M7-0109 | F3 Tower 323 |

Day 1:

* Streak isolates onto BHI agar plate
* Incubate at 32°C overnight (16-18 h)

Day 2:

* Inoculate 5 mL BHI broth with a single colony
* Vortex for 5 seconds
* Incubate tube at 32°C overnight (16-18h)

Day 3:

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

Day 4:

* Enumerate cfu on BHI plates from your overnight culture and record in excel spreadsheet.
* Adjust with PBS to achieve an inoculum concentration of 100 cfu/mL using the following calculation:
* = mL media needed
* Prepare inoculums: resuspend the desired culture (1 x104 cfu in 10 mL) in media (SMB, UHT, milk). Transfer 1 mL of resuspended culture to 9 mL of uninoculated media, for a total of 3 tubes per media tested (so 9 tubes in total). Vortex for 5 seconds to resuspend.
* Remove 500 uL for plating and, using the spiral plater, spiral plate both an undiluted culture and the 10-1 dilution onto BHI plates in duplicate using the exponential 50 setting.
* Incubate inoculated milk tubes at 6°C, and the spiral-plated agar plates at 32°C overnight (16-18 h). Be sure to include 3 uninoculated control tubes (1 for each of the media) which will be used to account for potential contaminants.

Day 5

* Enumerate starting inoculum using the Q-count, to ensure the inoculum was 100 cfu/mL. Record results in excel spreadsheet.
* If the inoculum was between 90 – 200 cfu/mL the tubes will be incubated for 21 days, if not, repeat days 2-5.

Day 18

* Remove tubes from the 6°C incubator and vortex for 5 seconds.
* Aseptically transfer 500 uL of cultures into 1.5 mL Eppendorf tubes. Return tubes to 6°C incubator.
* Perform serial dilutions to 10-5.
* Plate undiluted, 10-2, and 10-4 dilutions (50 uL on 50-exponential setting).
* Incubate inoculated plates at 32°C for 24 hrs.

Day 19

* Enumerate cfu on inoculated plates and record results in excel spreadsheet.

Day 25

* Remove tubes from the 6°C incubator and vortex for 5 seconds.
* Aseptically transfer 500 uL of cultures into 1.5 mL Eppendorf tubes. Return tubes to 6°C incubator.
* Perform serial dilutions to 10-5.
* Plate undiluted, 10-3, and 10-5 dilutions (50 uL on 50-exponential setting).
* Incubate inoculated plates at 32°C for 24 hrs.

Day 26

* Enumerate cfu on inoculated plates and record results in excel spreadsheet.
* Perform data analysis to determine if there is a difference in growth among the 3 media for the different bacterial genera.

*At this point, we will need to decide whether or not we will perform another set of experiments (to get a total of 6 replicates per treatment) and try for a short report.*