

Shelf life experiment

Goal:

Our goal was to check the shelf life and durability of our bacterial solutions containing glycerol and find the optimal conditions in which the product should be stored in stores and in the user's home. We used a solution of 80% glycerol and 20% LB (with or without glucose) in order to allow the viscosity of our solution to be such that the product won't drip on the consumer's scalp.

Method:

Tubes mimicking the solutions that we expect to incorporate in the final product were prepared. We examined the durability and lifespan of each bacteria in the 80% glycerol, 20% LB solutions, with or without the addition of glucose, in the following temperatures:

- At 28°C
- At 4°C
- At -20°C

The addition of glucose was in order to check whether or not it helps the survival of the bacteria in the different environments.

Protocol:

Preparation of materials:

- 80% glycerol
 - Add 240 ml of 100% glycerol to 60 ml 100% LB and autoclave.
- Prepared agar plates with the appropriate antibiotics (CM for *E.coli* and Spec for *B.subtilis*)

Procedure:

1. Prepare *E.coli* and *B.subtilis* overnight starters:
 - a. Put 5 ml of LB in a 50 ml falcon with 5 μ l with the appropriate antibiotics
2. Dilute the solution (1: 100) into a 500 ml Erlenmeyer: 1ml of solution into 100 ml LB
3. Grow the bacteria until 0.6 O.D in spectrophotometer
4. Transfer 45 ml from each of the bacterial solutions into two 50 ml falcons (4 in total)
5. Centrifuge for 10 min at 5000 rpm and 4°C, discard the flow through
6. Add 10 ml of glycerol 80% solution and vortex to break the pellet
7. Add additional 35 ml 80% of glycerol 80% solution

8. Add 0.9 ml glucose to one of the two falcons of each bacteria
9. Add 9 μ l of the suitable antibiotic to each falcon (1:1000)
10. Transfer 14 ml from each 50ml falcon into a new 15 ml falcon, twice
11. Transfer from each 50 ml falcon 300 μ l to Eppendorf's (about 10 Eppendorfs each). (because of the high viscosity of the liquid, we recommend to take the sample slowly) and plate
12. Divide samples as follows-
Temperatures: 28°C, 4°C, -20°C
 - *E.coli* + glucose
 - *E.coli*
 - *B.subtilis* + glucose
 - *B.subtilis*
13. Take a sample from each type of solution and temperature: 120 μ l (because the high viscosity of the liquid, we recommend to take the sample slowly) and plate. Take one at time zero and others each day at the same time (about 24 hours gap), for as long as you wish to run the experiment (we chose 5 days).

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