Bacterial Strain Preservation

BacterialStrainPreservation.doc
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INTRODUCTION

The method described is to preserve bacteria strains of *Pseudomonas aureofaciens* and *Pseudomonas chlororaphis* in 15% glycerol solution for long-time storage in a -80°C freezer. The purpose of storing strains of bacteria in a -80°C freezer is to have archives of newly acquired or created strains.

SUPPLIES NEEDED

- Fresh liquid bacteria culture
    - Grown overnight (at least eight hours)
- Sterile 60% glycerol solution (autoclave)
- micropipette
- 2 sterile pipette tips
- sterile micro centrifuge
- liquid nitrogen
- -80°C freezer

ROUTINE PROCEDURE

Making 60% Glycerol Solution

1) The solution is stored in the refrigerator.
2) If solution is low, make more by using a purple screw cap 100 ml media stock bottle to store the solution.
3) Using a graduated cylinder, measure 30 ml of Glycerol. Pour into the media bottle.
4) Rinse the graduated cylinder with DI water and then fill to 20 ml of DI water. Pour into the media bottle. The total volume of solution should be 50 ml.
5) Vortex or just swish the solution well and have it autoclaved.
6) Store solution in the refrigerator.

Making Fresh Liquid Bacteria Culture (Inoculation)

P. aureofaciens + P. Chlororaphis

1) Use flamed loop to transfer a single colony of *P. aureofaciens* from plate #2 or #3 to 5 ml of nutrient broth in a sterilized culture tube.
2) Leave on shaker overnight

Autoclaving

1) Autoclave as part of a larger batch for efficiency at 121°C for 30 minutes.
    a. Located in Johnson Hall room 227
    b. Autoclave should be set to 'liquid 60'.
2) Supplies to autoclave include:
    a. Micro centrifuge tubes
    b. Pipette tips

c. 60% glycerol solution
d. Include other supplies related to method for efficiency (ie: Petri plates, etc)

Making the Bacteria Archive

1) Label a sterile micro centrifuge tube with the date and name of bacteria
2) Pipette using a sterile pipette tip 0.1 ml of **sterile 60% glycerol solution** into the tube.
3) Using a new pipette tip, add 0.3 ml of the bacterial culture (frozen stock will be 15% glycerol). Use a new pipette tip for each bacteria strain.
4) Cap the tube and shake to mix the bacteria culture and the glycerol solution together.
5) Using a cooler filled with liquid nitrogen, immediately place the tubes inside to flash freeze the bacteria stock.
6) Bring cooler to Oceanography building (Marine Sciences) room 207 where the -80°C freezer is located and store tube in the freezer.
7) Remember to bring back the cooler.

Retrieving Bacteria Culture from Freezer Stock

1) In the Oceanography building (Marine Sciences) room 207/257, the stock tubes of the bacteria strains are kept in a -80°C freezer.
2) Label a Petri plate with bacteria species and date.
3) Use a sterilized toothpick to streak cells (plate #1).
4) Incubate the plate in the bacterial incubator located in room 303B.
5) Two to four days later, transfer a single colony by flamed loop from plate #1 to new plate and streak (plate #2).
6) Two to four days later, transfer a single colony by flamed loop from plate #2 to new plate and streak (plate #3).
7) Use only plate #2 and plate #3 for inoculation.

TROUBLESHOOTING

1) If you mess up start over.

REFERENCES

1) *Long Term Storage of Bacterial Strains*, Nov. 14, 1990. Donis Keller Lab, C. Helms.
2) *Bacterial Glycerol Stocks*, University of North Carolina.
3) *Glycerol Stocks of Transformed Cells*, South African Structural Biology Initiative.