Bacteria related jobs

TSA+NO3 plates:

1. Put 16g TSA + 0.4g KNO_3 into a 500ml media bottle, add 400ml DI water.
2. Autoclave (122ºC) for 30 minutes.
3. Pour plates and let sit overnight for solidification.
4. Wrap with parafilm and store upside-down in refrigerator.

Reviving bacteria cultures:

1. Label plate with bacteria species and date.
2. Use a sterilized toothpick to remove a dollop of frozen cells and spread immediately on the plate.
3. Use flamed loop to streak cells (plate #1).
4. Incubate the plate in the mass spec for p. aur; on top of mass spec for p. chl.
5. 2-4 days later, transfer a single colony by flamed loop from plate #1 to new plate and streak (plate #2).
6. 2-4 days later, transfer a single colony by flamed loop from plate #2 to new plate and streak (plate #3). On the same day, repeat steps 1-4 for reviving plate #1.
7. Only use plate #2 and plate #3 for inoculation.

Example schedule:
Thursdays – streak plates #1 and #3
Mondays – streak plate #2

Medium:

1. Weigh ingredients into 2.5L bottle (60g TSB, 2g KNO_3, 10g K_2HPO_4, 4g $(NH_4)_2SO_4$).
2. Add 2L DI water and stir until completely dissolved.
3. Distribute media into desired bottles (put 400ml in 500ml Pyrex media bottles and 130ml in 160ml serum bottles).
4. Keep cap loose on media bottles. For serum bottles, seal with septa and Al cap, then poke a vent needle through top of septum.
5. Autoclave (122ºC) for 1 hour.
6. Tighten all caps and remove all vent needles. When cool, label with ingredients and prep date and store in bacteria lab cabinet (away from sunlight and temperature variations).

Inoculation:

P. Aureofaciens

1. Use flamed loop to transfer a single colony of p. aur from plate #2 or #3 to 5ml of nutrient broth in a sterilized culture tube.
2. Leave on shaker overnight.
3. Use sterilized syringe and needle to inject 0.8-1ml into each serum bottle. Use media that is at least 1 week old. Shake well.
4. Label with species, plate date, inoculation date.
5. Log in blue book of bacteria/plate log.
6. Put bottles under mass spec; be sure to shake them up at least once each day.
7. Bacteria are ready for harvest 6-8 days after inoculation. Do not use after 10 days.

P. Chlororaphis

1. Use flamed loop to transfer a single colony of p. chl from plate #2 or #3 to 400ml medium bottle. Be sure to use media that is at least one week old.
2. Label with species, plate date, inoculation date.
3. Log in blue book of bacteria/plate log.
4. Put bottles on shaker standing upright.
5. Bacteria are ready for harvest 6-8 days after inoculation. Do not use after 10 days.

** Note: I was successful in growing p. chl in the serum bottles, using the protocol for p. aur inoculation. (Serum bottles will produce more vials per run.)

Harvesting:

** Nitrite test: to 5ml of media add 100uL of NED and 100uL of sulfanilamide. If media turns pink, nitrite is present in media and the bacteria are dead or wacky.
** Nitrate test: use fish tank test kit in bacteria lab.

1. Open all bottles and smell. If it smells strongly (often of chlorine), test for nitrite/nitrate.
2. Add 40ml of media to each centrifuge tube and centrifuge (18º, 10 min, 7500g). The bacteria plug should be dime-sized and pink.
3. Using an autoclaved pipette tip, reconstitute the bacteria plug in 4ml of media. Dump the rest of the media back in the serum/media bottle.
4. Add the 4ml aliquots together into two tubes, add a few drops of Antifoam B, mix well
5. Add 2mls of media to each 20ml headspace vial and cap. Put a long blue needle in each vial for venting.
6. Invert vials and purge through brown needles
   -- p. aur: 4.5 to 5 hours
   -- p. chl: 3 hours
7. Remove blue needle first, then vial from brown needle.
8. Add sample, taking care to rinse syringe and needle thoroughly with DI water between each sample. NEVER touch needle to sample. Always pull up sample with syringe only.
9. Rinse centrifuge tubes with ethanol/methanol. Rinse 3x with DI and dry in hood. Do not mix p. aur tubes with p. chl tubes.
10. Please check that there are enough for the next harvest:
    -- Clean vials
    -- Vial caps
    -- Needles
    -- At least 500psi He in tank
    -- Autoclaved pipette tips

Clean up:

1. Waste media: autoclave (20min) and flush down drain with water.
2. Waste vials: autoclave media (20min), neutralize, then flush down drain with water.
3. Waste solids and plates: autoclave in autoclave bag with indicator tape (gravity, 20min), then put in garbage.
4. Vials and media/serum bottles: soak in soapy water to clean, acid bath overnight, rinse well with DI water (or water bath overnight). Dry. Wrap in foil; muffle 500º for 4 hours.
5. Centrifuge tubes: Rinse with ethanol/methanol. Rinse 3x with DI and dry in hood. Do not mix p. aur tubes with p. chl tubes. Autoclave with caps loose every 2-3 weeks.