Growing Pseudomonas Aeruginosa for OD measurement and CFU calculations

Protocol Notes [1]

- Pseudomonas aeruginosa is a biosaftey level 2 pathogen. Be sure to open cultures in a biosafety hood ONLY.
- Be sure to work in a sterile environment at all times.
- The machinery used in this procedure needs to be warmed up and calibrated ahead of time.
- Review and be familiar with the IBC safety manual and protocol

Materials

1. 1L sterile flask
2. Sterile Petri dishes
3. Pseudomonas #27853 Working Stock; Location 24-231 -80C freezer #2
4. Trypticase Soy Agar (TSA) BD Catalog #11043 order from ThermoFisher; Location 24-231 room temperature
5. Mueller Hinton Broth order from Fluka; Location 24-231 room temperature
6. MgCl2, CaCl2; Location bacteria culture room (concentrations?)
7. ddH20
8. 1.5 mL microcentrifuge tubes
9. 96 well plate; Location 24-231 room temperature
10. Regulator Incubator; Location: bacteria culture room
11. Shaking Incubator; Location: ultracentrifuge room
12. Spectrophotometer; Location: bacteria culture room
13. Spectrophotometer cuvettes; Location: bacteria culture room

Methods

Making TSA plates

1. Dissovle 20g TSA concentrate in 500mL ddH_2O and mix by swirling plate with heater, until powder completely dissolved.
2. Autoclave at 121C for 20 minutes. (IMED autoclave settings: easy cycle, liquid, 15 minutes)

[1] Created by Created by DC on 2014-09-09; modified by ALG 2014-09-23

3. After agar has cooled, pour into sterile Petri dishes quickly, before the agar solidifies.
4. Store at 4C

Making MHB

1. Dissovle 21g MHB concentrate in 1000mL ddH_2O and mix by swirling plate, until powder completely dissolved.
2. Also add 0.9 mL of $MgCl_2$ and 2.25 mL of $CaCl_2$.
3. Autoclave at 121C for 15 minutes. (IMED autoclave settings: easy cycle, liquid, 15 minutes)
4. Store at room temperature.

Culturing Pseudomonas aeruginosa

1. Turn on the shaking incubator and ensure it is set for 37C.
2. Pour 600ml of prepared MHB buffer into a 1L flask.
3. Thaw three tubes of Ps. aeruginosa working stock and load them into the 1L flask of MHB using a micropipette. Mix well by swirling.
4. Using a sterile transfer pipette, transfer some of the bacteria culture into a cuvette and a 1.5 mL microcentrifuge tube.
5. Cover the cuvette with parafilm and seal the microcentrifuge tube. Put the microcentrifuge tube on ice and leave it there for at least 5 minutes.
6. Put the culture in the shaking incubator set at 37C.
7. Steps 3 through 5 are to be repeated every 2 hours for 12 hours.

Measuring Optical Density (to be done every 2 hours for 12 hours)

1. Turn on the spectrophotometer and let it warm up for 5 minutes
2. After choosing the single wavelength setting, set the desired wavelength at 600nm.
3. Zero the spectrophotometer using a cuvette filled with MHB without bacteria
4. After the spectrophotometer has been zeroed, take the appropriate cuvette prepared in "Culturing Pseudomonas aeruginosa" (above), wipe the sides with Kim Wipes, and insert it into the spectrophotometer
5. Record the OD measured.

CFU Calculations (to be done every 2 hours for 12 hours)

1. Dry an agar plate by slightly leaving the lid open in an inverted position
2. To prepare serial dilutions, fill wells A2-A11 of a 96 well plate with 90uL of MHB buffer.
3. Take the cell culture microcentrifuge tube off the ice and transfer 100uL of the cooled culture into well A1.
4. Perform serial dilutions by transferring 10uL of culture of A1 to well A2 and mixing. Then, take 10uL of the mixture of well A2 and transfer it to well A3 and mix well by pipetting. Do the same for wells A3-A11. The end result should be the dilution of culture into concentrations of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10} for wells A2, A3, A4, A5, A6, A7, A8, A9, A10, respectively.
5. When the agar plate is dried, transfer 10uL of each of the dilutions from 10^{-3} to 10^{-10} to appropriate locations on the agar plate. There should be 8 spots on the agar plate after a single transfer of each of the selected dilutions.
6. Let the agar plate dry completely by leaving the lid open. (takes about 1 hour)
7. These steps are to be done every 2 hours for 12 hours. Use row B for hour 2, row C for hour 4, so on and so forth.
8. Wait to incubate the plates until the plates from hour 12 is prepared and dried. All the plates need to go in the 37C incubator at the same time. Read the plates after 12 hours of incubation.