Culturing *Serratia marcescens*

By Nicola Kriefall (thenicolakriefall(at)gmail.com), modified from [Mansfield biorxiv](https://doi.org/10.1101/640177) infection of *Aiptasia* plus details from Dr. Rachel M. Wright’s protocols (rachelwright8(at)gmail.com), additional growth notes from [Application Note](https://physiology.case.edu/media/eq_manuals/eq_manual_Shaker_Agitation_Rate_and_Orbit_Affect_of_Bacterial_Growth.pdf) titled ‘Shaker Agitation Rate and Orbit Affect Growth of Cultured Bacteria’

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**Materials**

* Bacteria (glycerol stock or on agar plate)
* Orbital shaker (CO-Z, Amazon; must be able to rotate between 150-250 rpm)
* Tube holder to place on shaker
  + Notes: just using a 15 mL holder, a lot of wiggle room, could use a more fitted holder
* Culture tubes, dual snap lid
  + Notes: starting with small 5 mL ones, may increase size later based on results. Dual snap lid allows aeration
* Luria broth (L1530, US Biological Life Sciences)
* Pipettes & tips
* Bleach
* 70% ethanol in squirt bottle
* Glycerol (30% in DI water, autoclaved)
* DI water
* Sterile petri dishes (100 mm x 75 mm, holds 30 mL of liquid)
* Flame for sterilization
* Wire or inoculation stick
  + Notes: just needs to be able to make a small loop & be sterilized
* Permanent marker
* Autoclave & autoclave materials (see Autoclaving 101 protocol)

**Protocols**

*Materials preparation*

1. Luria broth (LB) or Marine Broth (MB)
   1. Add 7.75 g of luria broth powder OR XX g of marine brother powder into 500 mL of DI H2O in 1 L bottle (adjust volume & proportions as needed)
   2. Swirl bottle to mix
   3. Autoclave, allow broth to cool to room temperature before inoculating bacteria
2. Agar or Marine Agar (MA) plates (use within 2 weeks)
   1. Add 7.75 g of agar OR marine agar into 500 mL of DI H2O (adjust volumes & proportions as needed)
   2. Swirl vigorously to mix or use a stir bar
   3. Autoclave
   4. While autoclave is running, turn on water bath & set to 55˚C, and label the bottom of your petri dishes with either ‘agar’ or ‘MA’ depending on your starting material, and the date
   5. Place autoclaved bottle into 55˚C water bath, give it at least 20 min to equilibrate
   6. Pour melted agar into bottom of petri dishes (smaller one) quickly but carefully
   7. Place the lid back on top of the dish, very slightly ajar to let air in but not dust, allow to cool until solid
   8. Parafilm or place in sterile dishes sleeve & tape, store in 4˚C until use

*Streaking agar plate with bacteria from glycerol stock*

1. Retrieve your stock & agar plates, bring to lab bench
2. Light fire on ethanol-soaked wick
3. Flame the wire loop to sterilize (should turn red/white)
4. Allow loop to cool for a few seconds
5. Dip the wire loop into stock
6. Spread the stock on the plate
7. Wrap all plates in parafilm
8. Place in 37˚C incubator overnight (no shaking)

*Starting with bacteria grown on an agar plate*

1. Ethanol cleanse your bench, gloves, and a 1000 µl pipette
2. Transfer 2000 µL of LB or MB to sterile 5-15 mL culture tubes, repeat this for the number of colonies you’ll be plucking
   1. Notes: too much LB volume inhibits growth, LB/MB volume should be up to ~15% of tube volume
3. Retrieve agar plate. On the bottom of the plate, draw a marker circle around the colonies you want to pluck and label them. Choose isolated colonies, *i.e.* not visibly touching any other colony spots
4. Take a sterile, small pipette tip (0.2-20 µl size) and gently touch tip to the chosen colony (use a different tip for each colony)
5. Drop pipette tip into culture tube holding sterile media
6. Place culture tube into holder on an orbital shaker
7. Turn orbital shaker on to 180 rpm, no timer (just ‘on’ position), place in 37˚C incubator overnight, note the time that you started the incubation
   1. Speed notes: 150-250 rpm is typical for growing cultures, increase speed or incubation time for higher cell densities
8. The next day, retrieve cultures and make sure nothing grew in the negative control (you don’t have to make a negative control every time)

*Determining cell density*

1. Retrieve tubes from incubator/shaker
2. Make a new tube that will be a sterile marine broth blank (2 mL)
3. Centrifuge tubes at 3000 rpm for 5 min (make sure the 5 min starts ‘at speed’)
4. Pour off supernatant into container that will be bleached
5. Resuspend bacterial pellet & blank tube in 2 mL sterile sea water to wash, pipetting up & down vigorously
6. Centrifuge again, still at 3000 rpm for 5 min, discard liquid waste into container that will be bleached
7. Resuspend bacterial pellet in 2 mL sterile sea water, can resuspend media blank in 2 mL of sterile sea water, again pipetting up & down vigorously
8. Choose OD600 setting on the Denovix instrument (Nanodrop)
9. Use the washed LB blank to blank Nanodrop
10. Record OD600 measurements, make sure to eject tips into container to be bleached
11. Use 10% bleach on a Kimwipe to clean off the Denovix, then clean it off with another Kimwipe with DI water

*Determining CFUs (colony forming units)*

*Preserving bacterial stock in glycerol*

*Cleaning up*

1. Lab materials (pipette tips, Petri dishes, etc.): place in a container with 10% bleach (1 part bleach, 9 parts water).
2. Liquid waste: place in a bottle to be autoclaved at the end of the day, do not fill containers more than ¾ full
3. Plate waste: biohazard bag from BU facilities