

Solutions:

Sterile 1 M CaCl₂ stock solution

Materials: 14.7g CaCl₂
100ml beaker
stir bar
empty 100ml bottle

Procedure: Weigh 14.7g of CaCl₂ and add to 70ml of water. Stir to dissolve. Bring volume to 100ml with polished water and stir to mix solution. Transfer to 100ml bottle and autoclave on media cycle (4) or liquid cycle (3).

TY Broth (for liquid cultures of *Sinorhizobium meliloti*)

Materials: Tryptone Peptone	5g	Under hood work: 1M CaCl ₂
Yeast Extract	3g	pipet tips
1L beaker		hot mitt
stir bar		ethanol (to spray hood)

Procedure: Add 5g of Tryptone Peptone and 3g of Yeast Extract to 1L of polished water. Autoclave on cycle 3. Under bio hood, add 10ml of sterile 1 M CaCl₂ to 1L of TY Media (CaCl₂ stock made and autoclaved separately, as above). Use bio hood protocol for flaming bottles and general practices of sterile technique. NOTE: Indicate on the label that calcium has been added.

TY Agar

Materials: Tryptone Peptone	2.5g	Materials for bio hood: 1M CaCl ₂
Yeast Extract	1.5g	pipet and tips
500ml beaker		hot mitt
stir bar		Ethanol (wipe hood)
Bacto Agar		5 flasks of TY solid
5 flasks		

Procedure: (1) Add 2.5g Tryptone Peptone and 1.5g Yeast Extract to 500ml of water and stir. (2) Weigh out 1g Bacto agar into each of 5 flasks. (3) Add 100ml TY soln to each flask. (4) Autoclave on media cycle 4 or liquid cycle 3. (5) Under bio hood, wipe surface with ethanol. (6) While flasks are still hot (either fresh from the autoclave or microwaved to melt agar), add 1 ml of sterile 1M CaCl₂ to each of the 5 flasks. Follow protocol for flaming flasks under bio hood and general practice of sterile technique. (7) When finished wipe hood with ethanol again. Store flasks in cabinet or pour plates. NOTE: Indicate on the label that calcium has been added.

Starting a plate culture of *Sinorhizobium meliloti* 2011

1. After autoclaving TY agar and adding calcium, pour plates in the biosafety cabinet.
 - A. Flame the lip of the flask about 10 seconds.
 - B. Pour TY agar slowly and steadily into labeled plates (you should get 5 plates per flask).
 - C. Top loosely with Petri dish lids and push toward the back of the hood to cool.
 - D. Repeat as necessary until you have enough plates.
2. When plate is cool, take glycerol stock of *S. meliloti* from the -80 freezer and place on ice. Take to the biosafety hood.
3. Dip bacterial loop in ethanol and flame thoroughly. Allow to cool completely.
4. Dip loop in glycerol stock and draw across the plate in three lines on one end. Flame the loop.
5. Cap the glycerol stock tube and take back to the -80 freezer.
6. Turn plate 90° clockwise. Place the loop at the end of the last line and draw across the media. Draw across two more lines below the first and then flame the loop.
7. Repeat step 6.
8. Repeat step 6 again, but on the last line, draw it down in a zigzag pattern toward the middle of the plate.
9. Put the lid on the plate and invert it. Place it in a 28°C incubator to grow. Check daily and place in 4°C refrigerator once single colonies have appeared in the last streaks. Plate will be good 1-2 months, then a new one should be prepared.

Starting a liquid culture of *Sinorhizobium meliloti*

Use TY broth that has been autoclaved and had the calcium (above)

1. Prepare sterile 250 mL flasks. Cap clean 250 mL flasks with foam stoppers and cover the top of the flask with foil. Put autoclave tape on the foil and autoclave on cycle 2 (solid cycle). Do not open outside the biosafety cabinet.
2. Under the hood, open the flask by removing the foil. Remove the foam stopper and put it face first into the foil cap – this will keep the bottom of the stopper sterile. Flame the lip of the flask.
3. Using a sterile serological pipet, transfer 50 mL of sterile TY + Ca into a sterile 250 mL flask. Flame the lip of the flask and the TY bottle before and after pipetting.

3. Dip the bacterial loop in ethanol and flame thoroughly. Cool completely.
4. Using the loop, pick up a single colony from the *Sinorhizobium meliloti* plate prepared above. Flame the lip of the flask then put the loop into the liquid and shake it back and forth to disperse the bacteria into the broth.
5. Remove the loop and flame it thoroughly. Flame the lip of the flask and replace the bung and foil.
6. Put the flask in a 28°C shaker incubator set to 220-230 rpm. Bacteria should grow to the proper level for inoculation within 24-36 hours. Spec bacteria at fixed wavelength of 600λ.