Laura's Methods

From labwiki

Detailed experimental procedures are described below that I used in my study of how stress impacts predator-prey dynamics using the nematode *C. remanei* and *E. coli* bacteria.

Contents

Nematode growth medium (1.0%, 500 ml)

a. In a 1 litre flask with a magnetic stirring rod, mix together in order, the following chemicals

• 488 ml DI

• 1.00 g NaCl

• 1.55 g peptone

• 1.50 g KH_2PO_4

• 0.33 g K_2HPO_4

• 5.00 g agar

b. Cover lid with aluminum foil and autoclave on wet cycle for 20 minutes (takes approximately 1.5 hours) and then add the following chemicals in order after agar has cooled to approximately 50°C

• 500 ul ampicillin

• 500 ul 1M CaCl Stock

• 500 ul $MgSO_4$ Stock

• 800 ul colesterol Stock

• 625 ul nystatic Stock

Stock solutions

1M CaCl Stock - 14.7 g CaCl in 100 ml sterile DI

MgSO4 Stock - 24.6 g MgSO4 in 100 ml sterile DI

Colesterol Stock - 5 mg/ml in 100% ethanol, stir overnight

Nystatic Stock - 10 mg/ml in 100% ethanol, keep refrigerated

Ampicillin stock (10 ml) - dissolve 1 g of ampicillin in 10 ml of sterile DI, keep refrigerated

Sterile *E. coli* broth (1000 ml)

• 980 ml DI

• 10.00 g tryptone

• 5.00 g yeast

• 5.00 g NaCl

after autoclaving cool and add 1000 ml of ampicillin stock solution

Wire platinum spatula

a. light Bunsen burner

b. hold a hollow glass rod in the flame of the Bunsen burner at a 45 degree angle until glass is malleable using heat resistant gloves

c. place 1 cm of a 3.5 cm platinum wire into hollow of glass

d. use pliers to pinch glass closed around the wire

e. let spatula cool

f. use a hammer to shape the tip of the wire as desired

Creating a one week old nematode stock plate

a. Place 50 pregnant adult females on a 6 cm Petri plate inoculated with 50 ul of 0.8 OD600 OP50 – GFP using a wire platinum spatula and a dissecting microscope. Flame sterilize spatula with an ethanol burner between transfers.

b. Plates are used one week later to remove male nematodes from for the experiment

Pouring cuvettes

a. Place 500 ml of 1% autoclaved agar in steamer to melt for approximately half an hour

b. Sterilize cuvettes and caps in UV GeneLinker (in Charles Lab, B1-371) for two STR cycles (energy, time = 180 seconds)

c. Remove agar from steamer and let cool. The oven should be set to 50°C to keep the agar warm if needed and place a bottle of autoclaved DI water in the oven

d. Add stock solutions according to procedure for making Nematode Growth Medium

e. Autoclave plastic pouring tube inside tin foil and open only under flow hood to prevent contamination

f. Whip flow hood bench with 70% ethanol

g. Set plate pourer (Wheaten Omnispense PLUS) to 2.5 ml on 41 revolutions per minute (RPM)

h. Attach a sterile, non-barrier pipette tip to the end of the plate pourer tubing

i. Pour one cuvette by holding the end of pipette tip a couple millimeters above the agar to minimize formation of bubbles. Discard cuvettes that have bubbles or a pronounced meniscus

j. Repeat step i. for the desired number of cuvettes

k. Run sterile DI water through plastic pouring tube to prevent agar from clogging it

l. Let cuvettes evaporate for 20 minutes under the flowhood before putting the caps on

Adding *E. coli* to cuvettes

a. Turn on flowhood and sterilize surface

b. _ _:00 Remove cultures from fridge and cuvettes from incubator

c. _ _:05 Bring *E. coli* culture to OD600 = 0.8 +/- 0.01 in a sterilized, 1 cm culture tube

d. _ _:10 Add 10 ul of *E. coli* culture to each cuvette in the flowhood. Use the 10-100 ul pipette tip, pressing as far as it goes (second stop) and discard the first aliquot into the culture tube by pressing to the first stop only. Draw a second aliquot and press to the first stop (there should only be one drop which will be attached to the pipette tip) and shake the pipette lightly to move the drop of culture to the very tip of the pipette. Apply the drop to the cuvette surface in the center, taking care not to puncture the agar.

e. Let the cuvettes evaporate with the lids off in the flowhood

f. _ _:55 Place the caps back on the cuvettes, label with the cuvette number (in order that *E. coli* was added)

g. Place cuvettes upside down in 20°C incubator, group by block

h. Repeat steps b-g for all time blocks

Moving male nematodes to cuvettes

a. _ _:00 Remove block of nine cuvettes from incubator and photograph using fluorescence microscope

b. _ _:05 Inspect cuvettes for irregularities such as bubbles or punctures made when adding E. coli and discard such cuvettes (so that eight cuvettes remain per block). There is one extra cuvette marked in the random order than can be used to replace a discarded cuvette.

c. _ _:10 Open and cover cuvettes with their caps.

d. For modified agar treatments, create a 1 mm puncture in the center of the agar. For nematode present treatments, move seven male nematodes onto the agar surface. Split number of nematodes moved so that the agar is touched three times. For nematode absent treatments mimic the disturbance caused by moving nematodes by touching the wire spatula to the surface of the agar three times. Do not unintentionally puncture the agar.

e. _ _:50 Seal the caps tightly back onto the cuvettes. Record how many minutes the cuvettes lids were removed for ("Time" on data sheet)

f. Return cuvettes upside down to incubator

g. Repeat for steps a. to f. for eight blocks

Applying stress

a. _ _:00 Turn on flowhood

b. Sterilize flowhood surface with ethanol

c. _ _:00 Remove cuvettes for the appropriate block from the incubator

d. Count number of nematodes on the sides of the cuvette (above agar surface) and record number

e. _ _:10 Take fluorescent photos of two clear sides of cuvette. Side “a” has a triangle at the top, side “b” does not. Take photos with the cuvette lid on the left (will be the opposite on the computer screen).

f. _ _:20 open lids of all 8 cuvettes under the flowhood and place in order

g. Sterilize the forceps with ethanol and lighter flame

h. Firmly apply a square filter paper to the appropriate high stress treatment cuvettes in order (place paper in sideways, then tilt up to cover surface of agar, use a gentle s motion to press down in two directions)

i. _ _:25 Put lids of the cuvettes back on

j. Turn off flowhood and light

k. _ _:50 Turn on flowhood. Open all cuvettes

l. Remove filter papers using sterilized forceps, place in ethanol for later disposal. Take care not to damage the agar.

m. _ _:55 Recap cuvettes, weigh (tare to empty cuvette with lid), count number of worms remaining in the cuvette, and place back in incubator upside down for 24 hours

n. Turn off flowhood and light

o. Count number of nematodes remaining in all nematode present treatments

p. Weigh cuvettes standardized to an empty cuvette with a cap and record

q. Repeat steps a – p. until all blocks have been completed

Homogenizing the cuvettes

a. _ _:00 Remove cuvettes of the first time block from incubator and take fluorescent photos of each (two sides per cuvette)

b. Fill 24 sterile culture tubes with 4.95 ml of sterile saline and 8 with 4.50 ml of saline

c. Label culture tubes according to cuvette number and dilution. Dilutions for high stress treatments are labelled as 10-2, 10-4, 10-5, 10-6 and dilutions for low stress treatments are labelled as 10-2, 10-4, 10-6, 10-7 (these are not the calculated final dilutions used when estimating colony forming units per ml but are labelled in this manner for convenience during the homogenization procedure). Dilutions 10-2, 10-4, 10-6 are made in culture tubes with 4.95 ml of saline and 10-7 and 10-5 dilutions are made with 4.50 ml of saline

d. Flame sterilize metal stirring rod with ethanol and lighter flame

e. Aseptically add 7 sterile glass beads (3mm) to first cuvette followed by 1.0 ml of sterile saline solution using the 100-1000 ul pipette

f. Use paddle end of stirring rod to detach agar from the walls of the first cuvette. Run the stirring rod along each of the four edges of the cuvette and one diagonally through the middle of the agar. Make sure no agar is stuck to the rod when removing the it

g. Flame sterilize metal stirring rod

h. Vortex cuvette for 15 seconds upright than 15 seconds up-side-down on speed 8

i. Tap cuvette 10 times on bench to remove bubbles

j. Take two 50 ul aliquots of the homogenized cuvette, drawing from the bottom of the cuvette to prevent agar from blocking the pipette tip, and place in 10-2 dilution

k. Vortex 10-2 culture tube for 10 seconds on speed 8 then transfer 50 ul aliquot to 10-4 tube

l. Vortex 10-4 culture tube for 10 seconds on speed 8 then transfer 50 ul aliquot to 10-6 tube

m. For high stress treatments vortex culture tube 10-4 once and transfer 500 ul aliquot into 10-5 tube. For low stress treatments vortex culture tube 10-6 once and transfer 500 ul into 10-7 tube

n. Flame sterilize metal stirring rod with ethanol and lighter flame

o. Repeat steps e-n for each cuvette in the block

p. Label 16 Petri plates with the appropriate cuvette number and dilution (10-6 and 10-7 for high stress, 10-7 and 10-8 for low stress)

q. Plate bacteria using the appropriate culture tube by vortexing then pipetting 100 ul onto the surface of an agar petri. For example, to plate 10-6 take 100 ul from the 10-5 culture tube. Spread the bacteria using a sterilized glass hockey stick twice around the plate but do not spread to the edges of the plate. Start with the highest dilution (least amount of bacteria) and use the same pipette tip and the same sterilized hockey stick. Sterilize the hockey stick after plating both dilutions.

r. Repeat step q for all cuvettes in the block (total of 16 plates with 2 plates per cuvette)

s. Let plates evaporate upright then place in empty sleeve and into 30°C incubator

t. Repeat steps a-s until all temporal blocks are completed

u. Incubate plates for 23 hours at 30°C

Saline solution 0.9% (15 ml per cuvette)

• dissolve 9 g of NaCl in 1000 ml of sterile DI

Dilution series

Bacteria, *sterile saline*

100 ul from cuvette into *4.95 ml* = label as 10-2

50 ul from 10-2 into *4.95 ml* = label as 10-4

500 ul from 10-4 into *4.50 ml* = label as 10-5 *high stress - plate 0.1 ml*

50 ul from 10-4 into *4.95 ml* = label as 10-6 *high stress, low stress - plate 0.1 ml*

500 ul from 10-6 into *4.50 ml* = label as 10-7 *low stress - plate 0.1 ml*

Agar for colony plate count (1 L, 15 ml per plate)

• 10.00 g tryptone

• 5.00 g yeast

• 5.00 g NaCl

• 15.00 g agar

• 1000 ul of ampicillin stock solution after autoclaving and cooled to 50°C

Cleaning Nematode Stocks

a. Select a nematode culture that is overpopulated but has *E. coli* lawn remaining and does not have dauer larvae (i.e. population is not starved). The most appropriate plates for bleaching will have a high population of pregnant females

b. Pipette 0.5 ml of 5% bleach onto the plate

c. Gently slide the plate in a figure eight motion to distribute the bleach across the plate

d. Use a sterile spatula to detach nematodes from the agar surface if necessary

e. Tilt the plate to one side so that the bleach pools on one side

f. Pipette 0.05 ml of bleach onto a fresh Petri plate, inoculated overnight with 0.05 ml of OD600 *E. coli* culture

g. Wait for the bleach to absorb into the agar

h. Label petri plate with date bleached and store upside down at room temperature

i. Plate will be ready to harvest pregnant females from in 1-2 weeks, depending on the number of pregnant females in your old culture

E. coli culture

a. Remove old *E. coli* culture from the fridge, blank *E. coli* broth, and sterilized 16 mm by 100 mm culture tube (with metal cap)

b. Label the empty culture tube with the date and type of culture

c. Turn on Bunsen burner and flame sterilize loop by holding it at a 45 degree angle in the flame. Let the loop cool

d. Flame top of empty culture tube

e. Use pipette to add 10 ml of blank *E. coli* broth into empty culture tube

f. Vortex old *E. coli* culture

g. Transfer one loop of *E. coli* culture from the old broth to the new broth

h. Turn off Bunsen burner and put away with loop in the cupboard

i. Put the old *E. coli* culture and unused blank *E. coli* broth back in the fridge to be later autoclaved and then disposed of

j. Put the new *E. coli* culture on the shaker at 175 RPM overnight for 18 hours

k. Check the OD600 of the *E. coli* culture using a 10 mm culture tube filled with 2 ml of the broth. If the OD600 is less than 0.800, then shake the culture for an additional hour or until the OD reaches 0.800

l. Put the *E. coli* culture in the fridge for later use

Microscope camera settings

• Take photo of side with arrow first (a) then side without arrow (b)

• Take two photos of clear side of cuvette only

• Lid of cuvette on the photographer's left

Brightness -0.50

Contrast 1.00

(Symbol that looks like a line plot) 1.00

Exposure 1.20 s, 122%

Colour offset -2.00

Saturation 0.2

Framestart 0/168

Framesize 1384/720

4.8 zoom

48.0 mm Field of View (FOV)

0.3x lense

Summary of experiment

a. Synchronize age of nematode stock plates

b. Fill cuvettes with 1% agar and store upside down in 20°C incubator

c. Add 10 µl of OP50-GFP and incubate for 24 hours at 20°C

d. Place 7 male nematodes on each cuvette

e. Incubate for 4 days at 20°C

f. Add filter paper and remove for high stress treatments

g. Incubate for 24 hours at 20°C

h. Homogenize cuvettes, perform dilution series, and plate bacteria

i. Incubate at 30°C for 23 hours

j. Photograph plates and count CFUs using ImageJ