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Analysis of *C. elegans* reproduction, survival, and chemotaxis on different bacteria V.2

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Behavioural Genomics

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We use this protocol and it's working

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Abstract

Analysis of *C. elegans* reproduction, survival, and chemotaxis on different bacteria types (described here, induced and uninduced *Agrobacterium* and *E. coli* (OP50).

Materials

Yeast Extract Peptone (YEP) agar:
1% Bacto peptone, 1% Yeast extract 0.5% NaCl, 1.5% BioAgar

Yeast Extract Peptone (YEP) media:
1% Bacto peptone, 1% Yeast extract 0.5% NaCl

Induction Media:
1% NH_4Cl, 0.145% $MgSO_4$, 0.15% KCl, 0.01% $CaCl_2$, 0.0025% $FeSO_4$-$7H_2O$, 2 mM $NaPO_4$ (pH 5.6), 50 mM 2-(4-morpholino)-ethane sulfonic acid (MES), 0.5% glucose and 100 µM Acetosyringone

LB-Nematode Grown Media (NGM) Agar: (dx.doi.org/10.17504/protocols.io.b4c2qsye)
1% tryptone, 0.5% yeast extract, 1% NaCl, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 1 mM KPO_4
dx.doi.org/10.17504/protocols.io.bnh2mb8e, 1.7% bioagar, 5 µg/mL Cholesterol

NGM Agar: (dx.doi.org/10.17504/protocols.io.bvh7n39n)
0.3% NaCl, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 1 mM KPO_4, 0.25% Bacto Peptone, 1.7% BioAgar, 5 µg/mL Cholesterol

LB Broth:
1% tryptone, 0.5% yeast extract, 1% NaCl

LB Agar:
1% tryptone, 0.5% yeast extract, 1% NaCl, 1.5% BioAgar

Preparation of *C. elegans*

1 3 days before starting assays, bleach synchronize N2 *C. elegans*.
dx.doi.org/10.17504/protocols.io.2bzgap6

2 2 days before starting assays, refeed half the worms onto Nematode Growth Media (NGM) agar plates seeded with *E. coli* (OP50), keep at 20°C for 2 days (for the worms to reach L4 stage).

Preparation of *Agrobacterium*

3 5 days before starting the assays, streak *Agrobacterium* to Yeast Extract Peptone (YEP) agar plates and grow for 3 days at 28°C.

4 At 2 days and 1 day before starting the assays, innoculate 20 ml YEP media with a single colony of *Agrobacterium* and incubate overnight at 28°C and 250 rpm.

4.1 To prepare uninduced *Agrobacterium,* stop at this step. Note: start the cultures for non-induced *Agrobacterium* one day later than for induced *Agrobacterium*, so that cultures are ready at on the same day.

5 1 day before starting the assays, dilute overnight cultures to be induced 1:100 with YEP media, and culture at 28°C at 250 rpm until the OD600 reaches 0.3.

6 Pellet *Agrobacterium* by centrifugation (5 min 4000g), before resuspending in 1 ml Induction Medium containing 100 µM Acetosyringone. Shake at 50 rpm at room temperature for 23 hours.

7 On the day of the assays, pellet both induced or uninduced agrobacterium and resuspend in LB broth to a final OD600 of 1.8. (Note: LB broth was chosen as the media for resuspension for *Agrobacterium* and *E. coli* to keep conditions on the plates constant between bacteria types)

Preparation of *E.coli*

8 2 days before starting the assay, streak *E. coli* (OP50) to LB agar plates, and grow overnight at 37°C.

9 1 day before starting the assay, innoculate 10 ml LB broth with a single cology of *E. coli,* and grow overnight at 37°C 250 rpm.

10 On the day of the assays, pellet the *E. coli* and resuspend in LB broth to a final OD600 of 1.8.

Progeny Assay

11 Seed 90 mm LB-NGM plates with 1 ml of either *E. coli* , uninduced *Agrobacterium*, or induced *Agrobacterium* (normalized to an OD600 of 1.8).

11.1 Note: No antibiotics was included in LB-NGM plates to keep conditions between bacteria types constant and remove effects of antibiotics on *C. elegans.*

12 Transfer approximately 50 synchronzed L1 N2 *C. elegans* to the seeded LB-NGM plates. Record the exact number of worms dispensed to each plate.

13 Monitor the plates every 24 hours and record the number of eggs and progeny on each plate.

13.1 Monitor the plates every day untill F2 generation eggs appear.

14 Caluclate the number of eggs and progeny per worm by dividing by the total number of worms per plate.

Lifespan Assay

15 Seed 90 mm LB-NGM plates (containing 400 µM 5-Fluorodeoxyuridine) with 1 ml of either *E. coli*, uninduced *Agrobacterium*, or induced *Agrobacterium* (normalized to an OD600 of 1.8).

15.1 Note: No antibiotics was included in LB-NGM plates to keep conditions between bacteria types constant and remove effects of antibiotics on *C. elegans.*

16 Transfer approximately 50 synchronzed L4 N2 *C. elegans* to the seeded LB-NGM plates. Record the exact number of worms dispensed to each plate.

17 Monitor the plates every 24 hours, and record the number of dead worms per plate.

17.1 Classify worms as dead if they fail to repsond to a gentle touch to the head using an eyebrow pick.

18 Calculate the percentage of surviving worms.

Chemotaxis assay

19 Add a droplet of 100 µL of *E. coli* or induced *Agrobacterium* at opposite ends of a 90 mm LB-NGM plate. (Both cultures are normalized to an OD600 of 1.8).

19.1 Note: No antibiotics was included in LB-NGM plates to keep conditions between bacteria types constant and remove effects of antibiotics on *C. elegans.*

20 Transfer approximately 40 synchronzed L4 N2 *C. elegans* to the centre of the LB-NGM plates.

21 After 4 hours, count the number of worms on each bacterial lawn, and calculate the Chemotaxis Index (CI) (number of worms on *E. coli* - number of worms on *Agrobacterium*/ Total number of worms).