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Analysis of *C. elegans* reproduction, survival, and chemotaxis on different bacteria V.1

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Protocol status: Working

We use this protocol and it's working

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Abstract

Analysis of *C. elegans* reproduction, survival, and chemotaxis on different bacteria (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% NH₄Cl, 0.145% MgSO₄, 0.15% KCl, 0.01% CaCl₂, and 0.0025% FeSO₄-7H₂O, 2 mM NaPO₄ (pH 5.6), 50 mM 2-(4-morpholino)-ethane sulfonic acid (MES), 0.5% glucose + 100 µM Acetosyringone

LB-Nematode Grown Media (NGM) Agar:

1% tryptone, 0.5% yeast extract, 1% NaCl, 1mM CaCl₂, 1mM MgSO₄, 1mM KPO₄

[dx.doi.org/10.17504/protocols.io.bnh2mb8e](https://doi.org/10.17504/protocols.io.bnh2mb8e), 1.7% bioagar, 5 µg/mL Cholesterol

NGM Agar:

0.3% NaCl, 1mM CaCl₂, 1mM MgSO₄, 1mM KPO₄, 1.7% bioagar, 5 µg/mL Cholesterol

LB Broth:

1% tryptone, 0.5% yeast extract, 1% NaCl

Preparation of *C. elegans*

- 1 3 days before starting assays, bleach synchronize N2 *C. elegans*.
[dx.doi.org/10.17504/protocols.io.2bzbgap6](https://doi.org/10.17504/protocols.io.2bzbgap6)
- 2 2 days before starting assays, refeed half the worms onto NGM 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 2 days and 1 day before starting the assays, inoculate 20 ml YEP media with a single colony of *Agrobacterium*, 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 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 growth conditions 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, inoculate 10 ml LB broth with a single colony 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).
- 12 Transfer approximately 50 synchronized 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 until F2 generation eggs appear.
- 14 Calculate 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 μ M 5-Fluorodeoxyuridine) with 1 ml of either *E. coli*, uninduced *Agrobacterium*, or induced *Agrobacterium* (normalized to an OD600 of 1.8).
- 16 Transfer approximately 50 synchronized 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 respond 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 μ L 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).
- 20 Transfer approximately 40 synchronized 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).