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Effect of supplementation of Iron and Enterobactin on C. elegans behaviour on Keio E. coli mutants (6-well plates)

Forked from Testing the effect of paraquat on C. elegans behaviour when on Keio E. coli mutants (6-well plates)

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Behavioural Genomics

Saul Moore

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ABSTRACT

Protocol for screening candidate behaviour-modifying *E. coli* BW25113 single-gene deletion mutants from the 'Keio Collection', to investigate their differential effects on *Caenorhabditis elegans* behaviour when supplemented with iron and enterobactin.

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FORK NOTE

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MATERIALS TEXT

For bacterial culture:

- 500mL LB
- 50mL Erlenmeyer flasks

For worm maintenance and imaging plates:

- 1L NGM agar (for ingredients, see protocol for making NGM agar)
- 60mm Petri plates ('maintenance plates')
- 90mm Petri plates ('nursery plates')
- 6-well flat bottom plates ('imaging plates')

Supplements:

- Iron(III)chloride ($FeCl_3$) reagent grade, 97% (157740-100G Sigma-Aldrich, CAS: 7705-08-0)
- Iron(III)sulphate ($Fe_2(SO_4)_3$ xH_2O) hydrate (307718-100G Sigma-Aldrich, CAS: 15244-10-7)
- Enterobactin ($C_{30}H_{27}N_3O_{15}$) from *Escherichia coli*, ≥98% (HPLC) (E3910-1MG Sigma-Aldrich, CAS: 28384-96-5)

- KIMTECH Science lint-free precision wipes

SAFETY WARNINGS

Iron and enterobactin are toxic substances, so ensure that you wear gloves and a lab coat when working with them.

DISCLAIMER:

Preparing NGM agar + pouring plates

1 Prior to screening, prepare the materials needed for screening *C. elegans* on selected Keio *E. coli* mutants:

- 6-well plates (aka. 'imaging plates')
- 15 mL Falcon tubes
- 50 mL Erlenmeyer flasks
- 90 mm Petri plates (aka. 'maintenance plates')

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- 150 mm Petri plates (aka. 'nursery plates')

2 Make 1L normal Nematode Growth Media (NGM) agar, following the protocol:

Making normal NGM for imaging plates (Cabreiro Lab)
by Saul Moore

3 Pour 15 mL NGM agar into each 60 mm maintenance plate, and 35 mL NGM agar into each 90 mm nursery plate, following the protocol for Plate pouring (dx.doi.org/10.17504/protocols.io.6bhhaj6).
Keep the remaining agar warm in a water bath set to 65°C, for pouring into 6-well imaging plates afterwards

4 Using the Integra ViaFill, dispense 4 mL NGM agar into each well of the 6-well plates, following the protocol:

Dispensing agar into multiwell plates
by Ida Barlow

5 Leave the plates on the lab bench (with lids on) until the agar has cooled and solidified (approximately 1 hour, timing depends on humidity)

6 Measure the weight of 3 imaging plates (with lids on) and record average plate weight on day of pouring

7 Dry the imaging plates under a hood (or drying cabinet) until the plates lose between 3-5% of their original plate weight (with lids on)

8 Store the imaging plates upside-down at 4°C until used for experiments

Preparing worms

Inoculate 10ml LB broth media with *E. coli* BW25113 (Keio background wild-type strain, used

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9 as negative control and for raising worms, no Kanamycin) in an Erlenmeyer flask for overnight culture following the protocol:

Inoculating a Liquid Bacterial Culture
by Priota Islam

10 Place the inoculation in a shaking incubator at 37°C at 200 rpm and leave to grow overnight

11 Remove the BW culture from the shaking incubator and place in 4°C fridge until seeding

12 Remove the plates from storage and the BW culture from the fridge, and leave on the bench for approximately 30 minutes to acclimate to room temperature

13 Using aseptic technique, seed the 60 mm maintenance plates each with approximately 250 µL of BW25113 culture

14 Leave under hood until dry (with lids on, timing depends on humidity)

15 Using a platinum pick, gently pick 30 adult N2 Bristol *C. elegans* onto each maintenance plate, and store in an incubator at 20°C

16 After 24 hours, remove the adult worms, leaving the eggs behind to hatch into L1 larvae

17 Inoculate a further 10 mL LB broth with BW25113 bacteria for overnight culture (no Kanamycin), following the protocol in and place in a shaking incubator at 37°C, 200 rpm

18 After 24 hours, remove the culture from the incubator, and the 90 mm nursery plates from

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storage, and leave to acclimate on bench top for 30 minutes

19 Seed the nursery plates each with approximately 1 mL of fresh BW25113 culture. Leave under hood until dry

20 Wash the worms off the BW-seeded maintenance plates, into two 15ml Falcon tubes

21 Perform an egg prep on worms in the Falcon tubes, following the protocol:

Egg Prep for Bleach Synchronization (Cabreiro Lab)
by Saul Moore

22 At around noon the next day, wash L1-arrested larvae off the empty plate and re-feed onto the BW-seeded nursery plates using a glass Pasteur pipette. Aim to dispense around 500 worms per plate.

23 Incubate at 20°C for 68 hours until the worms are Day 1 adults for the experiment

Preparing bacteria

24 Fill 2 separate Erlenmeyer flasks with 25 mL LB. Add 50µg/ml Kanamycin to one flask, and leave the other flask without Kanamycin for the BW25113 control.

25 Remove the required Keio frozen stock plates from -80°C containing the strains for antioxidant testing. Gently remove the aluminium film and leave to partially thaw for a minute or so

To avoid damaging the bacterial stocks through repeated freeze-thawing, do not let the wells completely defrost. Just enough to be able to pick up some cells with the replicator.

26 Inoculate the Erlenmeyer flasks with the desired strains for antioxidant testing from Keio frozen stock plates, following the protocol:

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Inoculating a Liquid Bacterial Culture
by Priota Islam

27 Incubate the cultures overnight at 37°C in a shaking incubator at 200 rpm.

28 Remove the overnight cultures from the incubator. Inoculate 2 more Erlenmeyer flasks for a second round of overnight cultures from the first, this time without Kanamycin (to avoid exposing the worms to the antibiotics), and incubate overnight at 37°C at 200 rpm.

29 After 24 hours, remove the cultures from the incubator and store at 4°C until used for experiments

Seeding imaging plates (6-well)

30 Remove the imaging plates from 4°C storage

31 Ensure that imaging plates have lost approximately 3-5% of their original weight (so that they are not too wet for imaging when seeded). Place under a hood or drying cabinet until they have.

32 Remove overnight cultures of Keio strains from 4°C storage. Using a pipette, seed 30 µL of bacterial culture into the wells of each 6-well imaging plate.

33 Place the seeded plates under a laminar flow hood to dry for 20 minutes, then place in an incubator at 25°C (no shaking) for 7 hours 40 minutes (total lawn growth time: 8 hours)

34 After 8 hours total growth time, remove the plates from the incubator and store at 4°C

Adding iron and enterobactin (6-well)

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35 On the day of tracking, remove the seeded imaging plates from 4°C, and dry for 30 minutes under a laminar flow hood

36 Remove the iron(III)chloride, iron(III)sulphate and enterobactin from 4°C.
- Prepare 100 and 400 mM of iron(III)chloride and iron(III)sulphate (in H_2O)
- Prepare 1 mg/mL enterobactin solution (in DMSO)

37 Using a pipette, dispense 40 µL of iron(III)chloride and iron(III)sulphate into the desired wells (on top of the lawns) of the 6-well imaging plates (for a final concentration of 1 and 4 mM iron in 4 mL agar)

38 Using a pipette, dispense 5 µL of enterobactin solution on top of the lawns of the desired wells in the 6-well imaging plates (for a final concentration of 1.25 µg/mL enterobactin in 4 mL agar)

39 Leave the plates to dry in a biosafety hood for 30 minutes.

40 Record the weight of the plates after drying (as weight at imaging).

41 Then leave the plates on the bench (with lids on) for a further 1 hour 30 minutes (total 2 hours with the supplements added) before adding worms.

Picking worms + Hydra tracking (6-well)

42 Prior to tracking, ensure that the imaging cave air conditioning is turned on (and there has not been a power-cut) and also empty the dehumidifier waste water tray (see pre-imaging checklist)

43 Remove the nursery plates of worms from the 20°C incubator.

44 Using a platinum worm pick, carefully pick 10 Day1 worms onto the edge of the bacterial lawns in each well of the 6-well imaging plates, then place in incubator at 20°C until tracking (after 4

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hours on food + supplements).

45 30 minutes prior to tracking with the Hydra rig (each run is performed every 20-30 minutes), remove 5 imaging plates at a time from the 20°C incubator and leave to acclimate in the imaging cave.

46 Wipe the underside of the lids using KIMTECH Science lint-free precision wipes (to remove any condensation that has formed)

47 Place the plates under the Hydra rig and record worm behaviour on the bacterial food for 15 minutes (at the 4-hour timepoint, 25 fps, exposure: 25000 msec, blue-light stimulation)

48 After tracking, discard the plates in a biological waste bin

49 Check tracking checklist to ensure that all videos have been saved correctly: '/Volumes/behavgenom$/Documentation/Protocols/analysis/tracking-checklist-20210210.docx'

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