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Testing the effect of *E. coli* cell supernatant and lysate on *C. elegans* behaviour

Saul Moore¹¹Imperial College London

1

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Behavioural Genomics



Saul Moore

Experiment to identify whether the behavioural effect on worms feeding on *E. coli* mutants from the initial screen of the Keio Collection are due to metabolite(s) present in either the cell supernatant or cell lysate. Cell cultures were grown on either solid NGM agar or liquid NGM media, and lysate and supernatant extracted from both culture types.

Treatments tested:

- Keio mutant and control bacteria supernatant vs lysate
- From solid vs liquid culture
- Added to live vs UV-killed bacterial lawns
- Solvents used: PBS/NGM/DMSO/methanol

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Keio, bacteria, supernatant, lysate, *C. elegans*, behaviour, ultraviolet

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To make 1L of Nematode Growth Media (NGM) agar:

- 3g NaCl
- 2.5g Bactopectone
- 17g Agar powder
- 1L ddH₂O

Salts added post-autoclave:

- 25mL KH₂PO₄ (pH=6.0)
- 1mL MgSO₄ [1M]
- 1mL CaCl₂ [1M]
- 1mL Cholesterol (5mg/mL in EtOH)

To make liquid NGM, follow the instructions for making NGM agar, then filter the agar before the media cools and solidifies.

Cell culture on solid media

- 1 25mL fresh LB broth was added to each of 2 Falcon tubes, and inoculated with BW25113 parent strain for the Keio Collection, and the *E. coli* gene-deletion mutant of interest, respectively.
- 2 The inoculations were left to grow overnight in a shaking incubator (37°C, 200rpm)
- 3 The next day, 120ul of each bacterial culture was added to 60mm Petri plates containing 15mL Nematode Growth Media (NGM) agar
- 4 The bacterial lawns were incubated at 25°C for 3 days
- 5 Afterwards, 1.5mL PBS (0.5X) was added to the lawns and the bacteria scraped off the NGM plates and put into Eppendorf tubes
- 6 The Eppendorf tubes were centrifuged for 2 minutes at maximum speed
- 7 The supernatant was separated from the pellet using a pipette, dried using a SpeedVac

machine, then resuspended in 50% DMSO ('Solid Supernatant')

- 8 Two methods were tried to produce the lysate solution from the remaining pellet in the Eppendorf (after the supernatant was extracted):
 - (1) the pellet was resuspended in 0.5mL PBS (0.5X) by vortexing, then sonicated ('Solid Lysate in PBS')
 - (2) the pellet was resuspended in 0.5mL methanol by vortexing, then sonicated ('Solid Lysate in methanol')

Resuspended pellets were sonicated for 10 minutes, then left to settle for 10 minutes, before a further round of sonication for 5 minutes (100% amplitude).

Cell culture in liquid media

- 9 25mL liquid NGM (NGM with agar filtrated) was added to each of 2 Falcon tubes, and inoculated with BW25113 parent strain for the Keio Collection, and the *E. coli* gene-deletion mutant of interest, respectively.
- 10 The inoculations were left to grow overnight in a shaking incubator (37°C, 200rpm)
- 11 The next day, the cultures were removed from the incubator and centrifuged for 2 minutes at maximum speed
- 12 The supernatant was separated from the pellet using a pipette, dried using a SpeedVac machine, then resuspended in 50% DMSO ('Liquid Supernatant')
- 13 The lysate was produced by resuspending the pellet in 0.5mL PBS (0.5X) by vortexing, and then sonicating ('Liquid Lysate')

The resuspended pellet was sonicated for 10 minutes, then left to settle for 10 minutes, before a further round of sonication for 5 minutes (100% amplitude)

Preparing bacteria for imaging

- 14 Prepare 2 x Erlenmeyer flasks with 50mL LB for inoculating a liquid bacterial culture of BW25113 control and the mutant bacteria to test. Add 50mg/mL Kanamycin to the mutant bacteria flask (the gene-deletion mutants have a Kanamycin resistance cassette in place of the deleted gene)

- 15 Inoculate from a single colony picked from streaked LB plates stored at 4°C, and leave overnight in a shaking incubator (37°C, 200rpm)
- 16 The next day, remove the bacterial cultures from the shaking incubator, and prepare another two Erlenmeyer flasks each with 50mL LB, for inoculating a second round of overnight cultures. Only this time, do not add Kanamycin to the mutant bacteria flask
- 17 Inoculate the second round of cultures by pipetting approximately 50µL bacterial culture from the first overnight culture. Leave in a shaking incubator overnight (37°C, 200rpm)
- 18 The next day, remove the overnight cultures from the shaking incubator, and store at 4°C
- 19 UV-sterilise half of the culture for both the BW25113 control and the test mutant bacteria, by exposing to UV-C (254nm) UV light for 10 minutes and repeat 6 times (total of 1 hour UV exposure, with a short wait between exposures). Leave at 4°C until seeding imaging plates (max 2 days)

Preparing 6-well plates for imaging

- 20 Prepare 1L NGM agar and pour 4mL into each well of 40 x 6-well plates. Leave them to dry under a hood, until the plates have lost between 3 - 5% of their weight at pouring.
- 21 Remove imaging plates from 4°C, and dry under a hood for 30 minutes to remove condensation. Remove the bacterial cultures from 4°C and leave on the bench for 30 minutes to acclimate to room temperature.
- 22 Pipette 30µL of bacterial culture into the centre of each well, taking care not to damage the agar with the pipette tip. Seed half of the 6-well plates with BW25113 control (half of these with UV-killed bacteria, half live bacteria), and the other half with the test bacteria (half of these with UV-killed bacteria, half live bacteria).
- 23 Leave the seeded plates to dry for 20 minutes under the hood, then transfer to a 25°C incubator and leave to grow for a further 7 hours and 40 minutes (for a total of 8 hours lawn growth time), before storing at 4°C for tracking the next day
- 24 On the day of tracking, remove the seeded plates from 4°C and dry under a hood for 30 minutes to remove condensation

- 25 Approximately 1 - 2 hours prior to adding worms and imaging, add 80ul of supernatant or lysate (from either solid or liquid culture) to the desired wells, as per the experimental design.

Imaging with worm tracking rig (Hydra)

- 26 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)
- 27 Remove the plate of age-matched (N2 Bristol, Day1 adult) worms from 20°C incubator
- 28 Using an eyebrow hairpick, gently but swiftly transfer 10 worms onto the edge of the bacterial lawn of each well in a single imaging plate at a time.
- 29 Quickly transport the 6-well plates to the imaging cave and place them under the rigs. Ensure that the plate is in the correct orientation for the recording so that the positions of each of the wells under the cameras is correct and matches the recorded treatment information in the metadata
- 30 Track worm behaviour on each well for a total of 36 minutes (at 25 fps), applying a 10-second blue-light stimulus at the 30th, 31st and 32nd minute timepoints