C. elegans and Automated Imaging:

A straightforward and quantitative platform for characterising microbiome-host interactions in vivo prior to rodent studies.

D Weinkove[2]; B Virk[1]; J Jia[1]; C Maynard[1]; A Raimundo[2]; C Manning[1]; I Cummings[1]; L Zhao[3]; F Tholozan[2]; C Saunter[2];

[1] Durham University, UK; [2] Magnitude Biosciences, UK; [3] Rutgers University, United States

The microbiome holds great promise for drug development. For biotechs to take full advantage of their rich dataset of candidate strains, robust in vivo models and methods are required . Using the non-mammalian animal model *C. elegans*, Magnitude Biosciences is a UK-based CRO that can support mode-of-action and efficacy/safety studies at the junction between identification of a bacterial candidate strain from a genomics screen and its testing in rodent studies.

Why Worms?

Relevant
- ~ 40% genes have human orthologs
- Track record in ageing, neurodegeneration, cancer, metabolism
- Transgenics as models of inherited diseases
- Indicator species for pesticide toxicity with good concordance for known toxicity in mammals

Efficient
- Small : 1-2mm worms grown in Petri dishes
- Fast: development and 2-3 weeks lifespan
- Nematode: No regulatory restrictions
- Ethical: Reduces mammalian testing

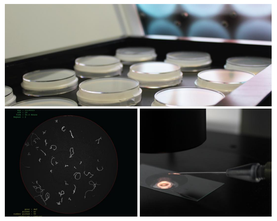
Top: *C. elegans* worm, showing internal organs. Bottom: Worm population maintained on bacterial lawn in standard Petri dishes. Phase contrast microscopy.

Versatile
- Nervous system, muscle, intestine, epidermis and reproductive system
- Transparent body: easy live visualisation of eg. GFP-tagged targets
- Amenable to transgenics, from strain banks or customised in-house.
- Assays for developmental toxicity or life-long intervention effects

Microbiome Testing
- *C. elegans* is routinely maintained on a bacterial lawn, which makes it ideal for studying host- bacteria interactions in the gut, either by direct co-culture with compatible strains, or by addition of bacterial extracts .
- *C. elegans* maintained in the lab do not have their own gut microbiome.

Revolutionary Technology

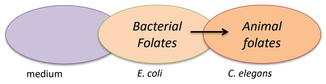
- Up to 32 Petri dishes at a time automatically tracked by separate small cameras each controlled by a single board computer.
- Near-continuously movement tracking: images taken every 0.8 seconds for 160 seconds, repeated every 5 minutes, for up to 10 days of worm adulthood.
- Non-invasive: no mechanical disruption, no abrupt changes in lighting or temperature.
- Multiple mobility parameters : worm speed, position, percentage moving, population fragmentation by speed, speed decline over time, chemotaxis, exploration, paralysis, increases in population size in fertile worms.
- Standardised reagents, protocols and schedules for manual worm maintenance prior to automated assays
- Assays monitored remotely.



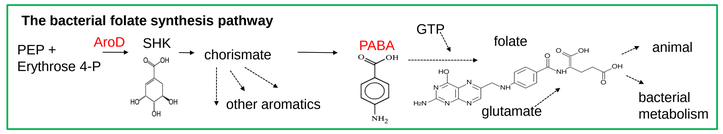
Top: Petri dish array set for illumination and image acquisition. Bottom Left: Representation worm tracks recording. Bottom Right: Micro-injection needle for transgenic strain generation.

Bacterial Mode-of-Action Case Study: *Bacterial Folate Synthesis Pathway and Host Lifespan*

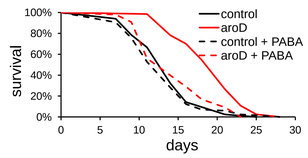
Background
Folates are important for human health but animals cannot make folates and rely on gut bacterial folate synthesis instead.

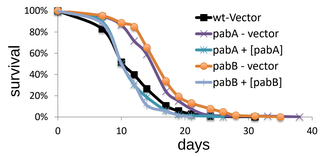


The bacterial folate synthesis pathway



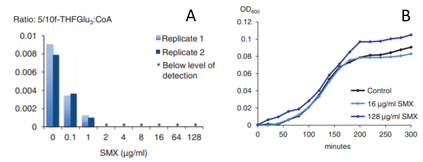
Disrupting bacterial folate synthesis increases *C. elegans* lifespan





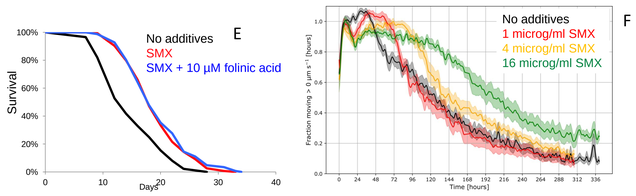
C. elegans worms fed on E.coli mutants with an inactive folate synthesis pathway have an increased lifespan. Addition of folate synthesis intermediates restores the lifespan to control levels. (Virk *et al.* 2012).

SMX disruption of bacterial folate synthesis increases lifespan



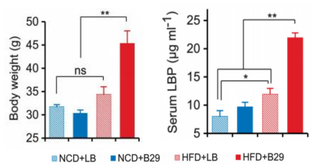
At a low dosage, the antibiotic sulfamethoxazole (SMX) blocks bacterial folate synthesis (A) without decreasing bacterial growth (B). At the same dosage, SMX increases C. *elegans* lifespan in a dose-responsive manner (C), without affecting fertility (D). Supplementation with nutritional additives maintain the increased lifespan (E). (Virk *et al.* 2012)

Automation of the assay also shows a delayed mobility decline in a dose-responsive-manner with SMX treatment, with greater sensitivyt of detection than the manual lifespan assay (F).



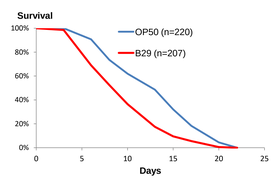
SMX has already been observed to increase rodent lifespan (Hackmann, 1958; Gsell 1962). Our C.elegans data mirrors this and provides a mechanism.

Human vs Mouse vs *C. elegans Case Study : Obesity-causing bacterial strain*



***Enterobacter cloacae* strain (B29), isolated from a morbidly obese human volunteer**, causes obesity in mice fed on a high-fate diet (HFD), but not in mice fed a normal chow diet (NCD). This correlates with an increase in serum LPS-binding protein (LBP), ,i.e. bacterial toxicity.

Data 16-weeks after inoculation.,- Advisor Board Member Liping Zhao Lab, (Fei & Zhao, 2013.).



***C. elegans* fed on B29 have a decreased lifespan compared to worms fed *on E. coli* (OP50).**
Data collected manually over 3 weeks.
Data from Weinkove lab in collaboration with the Zhao lab.

We believe that adoption of our C. elegans research services will boost microbiome pipeline productivity prior to rodent studies, and increase fundamental understanding of microbiome-host interaction mechanisms. For more info, www.magnitudebiosciences.com or email info@magnitudebiosciences.com