Role of Kinases in *Caenorhabditis elegans* Immunity Against *Pseudomonas aeruginosa* PA14

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Opportunistic Pathogen: *Pseudomonas aeruginosa*

- *P. aeruginosa* is a bacterium found widely in soil and water
- Causes more than 50,000 infections per year and is the sixth most common pathogen in U.S. hospitals [1]
  - Blood infections, pneumonia, and infections following surgery can result in serious illness and death
- Healthy people are also exposed to risk of ear and eye infections
- Hard to eradicate due to its multi-antibiotic resistance [1]
  - Serious threat as designated by the Centers for Disease Control and Prevention: Antibiotic/Antimicrobial Resistance Report [1]

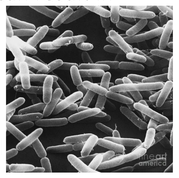


Fig. 1 *P. aeruginosa* [2]

Using *C. elegans* to Test Immunity Against *P. aeruginosa* Pathogenesis

- *C. elegans* is a tiny worm that provides us an excellent model for pathogenic studies
  - Susceptible to human pathogens [3]
  - Immune system is similar to human [3]

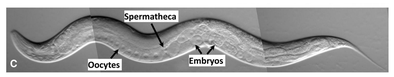


Fig.2 Observing *C. elegans* [5]

- *C. elegans – P. aeruginosa* Established Pathogenesis Assay
  - Slow Killing (SK) Assay
    - Most common method used on modified nematode growth medium (NGM) agar [3]
    - Death is caused by intestinal colonization and quorum sensing [3]
  - Liquid Killing (LK) Assay
    - Recently developed method conducted in liquid [3]
    - Death is caused by pyoverdine, an iron-binding siderophore secreted by *P. aeruginosa* [4]
    - Pyoverdine leads to mitochondrial damage and further hypoxic response [4]

Do Kinase Genes Hold the Key to Immunity?

- Prior research showed 5 kinase genes were upregulated in Liquid Killing
- All predicted to encode CHK-Kinase, which acts as a check-point during cell cycle [6]
- Project seeks to reveal if the 5 genes play a role in *C. elegans* immunity against *P. aeruginosa* infection under the different virulence mechanisms
- Could be means of developing patient immunity to *P. aeruginosa* infection

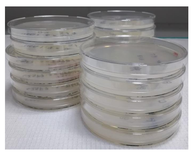
Table 1 Kinase Genes Upregulated in LK

Genes	Expressed in [6]
C29F7.2	Intestine, pharynx
F49C12.7	Unknown
F59B1.8	Unknown
T16G1.6	Head neurons, intestine
T16G1.7	Unknown

Methods: *C. elegans* Exposure to *P. aeruginosa*

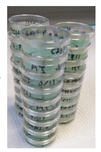
Knockdown of each kinase gene by RNA interference → Prepare *P. aeruginosa*

Grow RNAi-expressing *E.coli*



Grow *C. elegans* on *E. coli*

Slow Killing

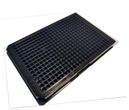


Incubate *C. elegans* with *P. aeruginosa* in 35 mm plates

Score dead worms using microscope each day

Liquid Killing

Sort *C. elegans* into 384-well plates by using COPAS worm dispenser



Incubate *C. elegans* with *P. aeruginosa*

Wash and stain with Sytox® dye

Image using automated microscope

Results: Slow Killing

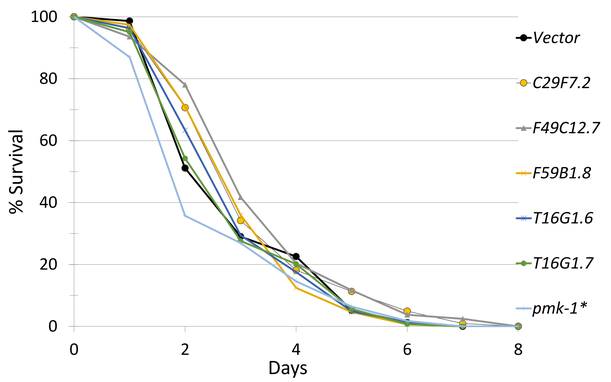


Fig.4 An example of *C. elegans* observed on a SK plate

pmk-1* was used as a positive control [7] (*p***=0.0392)

Fig.3 RNAi-treated *C. elegans* Survival Rate in Slow Killing

Among the 5 kinase gene knockdowns, only *F49C12.7* knockdown showed significant difference (***p*** = 0.0111) and died more slowly compared to the vector (control).

*Two more replicates were performed

Results: Liquid Killing

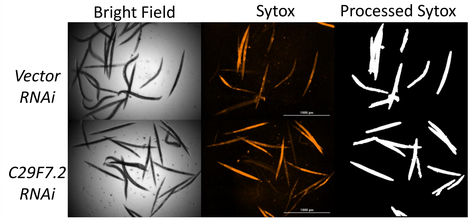


Fig.5
Images taken by using automated microscope Cytation® with the imaging software Gen5® 3.0. Images are processed and quantified by using the Cell Profiler® software

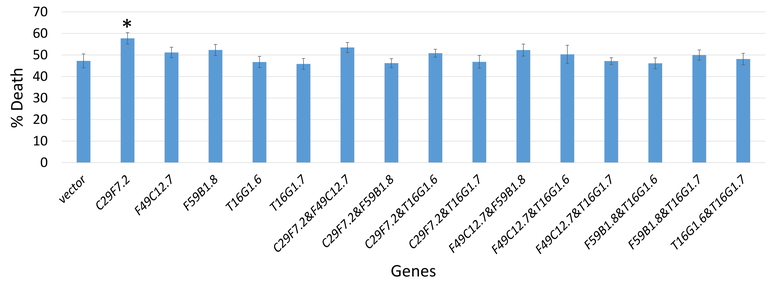


Fig.6 RNAi-treated *C. elegans* Death Rate in Liquid Killing

Among the 5 kinase gene knockdowns, *C29F7.2* knockdown (***p***=0.007643) showed significant increase in death rate compared to the vector (control).

*Two more replicates were performed

Discussion & Future Work

- A kinase gene *F49C12.7* is likely to be involved in *C. elegans* immunity against *P. aeruginosa* when infected through intestinal colonization, while *C29F7.2* is suggested to play a role when *C. elegans* is exposed to pyoverdine in Liquid Killing assay.
- Results inconsistency was observed in this study that might be due to weak RNAi phenotypes.
- Further studies are needed with more replicates and freshly spotted RNAi-expressing bacteria.

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