Using Survival Assays and RNA-seq to Identify Strain-Specific Differences in the Caenorhabditis elegans Response to Microbial Pathogens

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Abstract

Infection from microbial pathogens is a major threat to organismal survival. In its natural environment the microbivorous nematode, *Caenorhabditis elegans*, frequently encounters pathogenic bacteria. Although *C. elegans* possess physical barriers and exhibit coordinated behavioral responses to decrease the likelihood of infection, they must also recognize and respond to pathogens that have bypassed these defenses. This response is modulated through the innate immune system, a defense mechanism comprised of evolutionarily ancient components that are highly conserved across phyla. Yet, *C. elegans* do not exhibit obvious conservation of microbial defense pathways observed in arthropods and mammals, (*e.g.* Toll or NF-κB). Rather, pathogen detection occurs via many different systems that converge upon a core set of physiological responses as well as a set of pathogen-specific responses, some of which are conserved in other organisms (*e.g.* generation of reactive oxygen species, production of antimicrobial peptides, *etc.*). To investigate the evolutionary basis of innate immunity, we assessed survival of several *Caenorhabditis* strains infected with various pathogenic bacteria and found strain-specific responses to both *Pseudomonas aeruginosa* and *Enterococcus faecalis*. We are employing a two-pronged approach to identify molecular changes that may be responsible for these strain-specific differences in immunity. First, to detect transcriptomic changes we performed RNA sequencing of whole animals following 24 hours of pathogen exposure. This analysis yielded a large set of differentially expressed genes, some of which have been previously implicated in pathogen response. Currently we are curating a list of genes that are differentially expressed in both a pathogen-specific and strain-specific manner. Second, from our wild-type strains we generated crossbred F1s and compared survival of the cross-progeny to that of the wild-type strains following exposure to pathogenic *E. faecalis* and non-pathogenic *E. coli*. Genomic mapping of the F2 heterozygous offspring generated from these crossbred animals is underway to identify genetic loci overrepresented in animals exposed to *E. faecalis* relative to non-pathogenic *E. coli*. Ultimately, our study seeks to shed light on the evolutionary origins of innate immunity as well as reveal uncharacterized aspects of mammalian defenses against infection.

Methodology

Survival Assay:

30 late-stage L4 worms were placed on NGM plates seeded with microbial pathogen or non-pathogenic *E. coli* OP50. Worms were transferred to fresh plates daily and the number of dead worms was recorded. Four to seven biological replicates were collected per pathogen per worm strain.

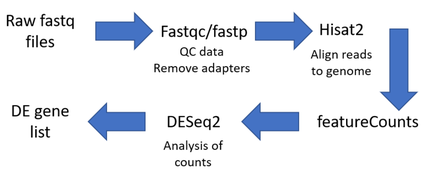
Exposure of Nematodes to microbes:

NGM plates were seeded with 250 µL of bacteria and incubated overnight at 37°C. Approximately 2,000 L4 stage worms were transferred to seeded plates and incubated at 20°C for 24 hours. After 24 hours, worms were resuspended in M9 buffer and mechanically disrupted in liquid nitrogen. Frozen tissue was transferred to a microcentrifuge tube, 1mL TRIZOL reagent was added, and tubes were flash frozen.

Preparation of RNA samples:

Sample were thawed and, after the addition of chloroform, centrifuged to concentrate RNA in the upper aqueous phase. Total RNA was extracted using the Monarch RNA Cleanup kit. RNA quality and integrity were assessed with Qubit and Agilent Tapestation. Sequence libraries were prepared using the TruSeq Stranded mRNA Library Prep Kit and sequenced using single-end 1x75 bp sequencing on the Illumina NextSeq 550 platform.

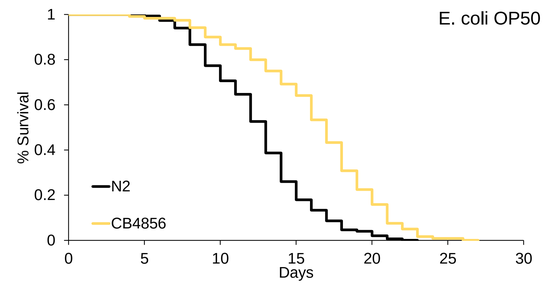
RNA-seq analysis:

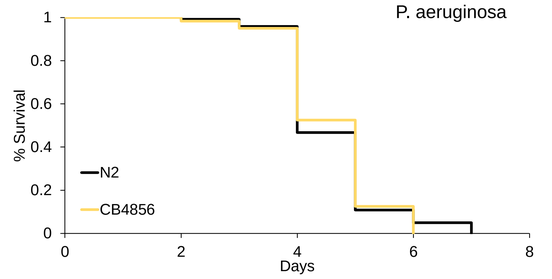


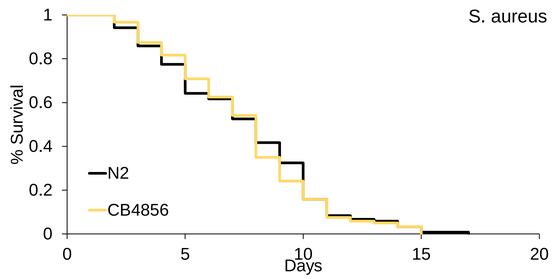
Bacterial Strains:

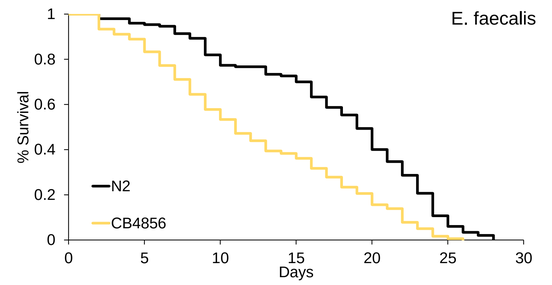
Species	Strain	Gram Stain	Pathogenicity
Escherichia coli	OP50	negative	non-pathogenic
Pseudomonas aeruginosa	PA-14	negative	medium-killing
Staphylococcus aureus	Newman	positive	medium-killing
Enterococcus faecalis	Andrewes & Horder	positive	slow-killing
Providencia rettgeri	Dmel1	negative	slow-killing

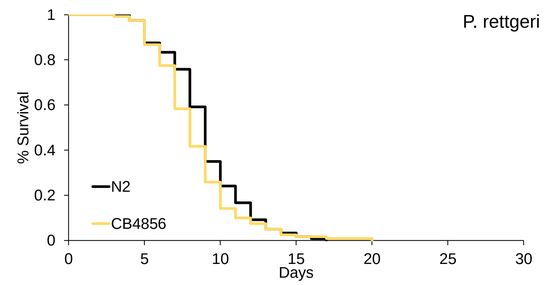
Caenorhabditis Species have Different Survival Times on non-pathogenic *E. coli* and Pathogenic Microbes





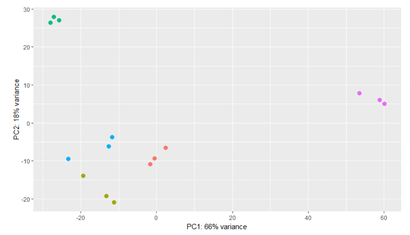




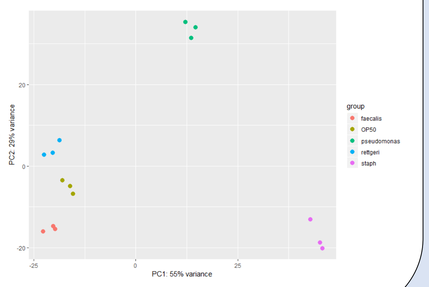


Principal Component Analysis Identifies Clusters of Samples Based on Pathogen Exposure

N2 Samples:



CB4856 Samples:



Summary of Differentially Expressed Genes

		Differentially Expressed Genes	
Worm Strain	Pathogen	Up-regulated	Down-regulated
CB4856	*P. aeruginosa*	1460	3711
	S. aureus	3597	3436
	E. faecalis	1296	480
	P. rettgeri	694	1559
N2	*P. aeruginosa*	1797	3995
	S. aureus	4891	3849
	E. faecalis	1754	630
	P. rettgeri	504	525

Future Directions

1.) Analyze RNAseq dataset to identify strain-specific and pathogen-specific differences in the response to microbial pathogen.

2.) Does geographic isolation of *C. elegans* influence response to microbial pathogen?
   a. Assay survival of geographically isolated strains of *C. elegans* in response to microbial pathogen
   b. Perform transcriptomic profiling of geographically isolated strains of *C. elegans* to identify strain-specific responses

C. elegans Strain	Location
N2	Bristol, UK
CB4856	Hawaii, USA
ED3040	Johannesburg, South Africa
ED3053	Limuru, Kenya
MY1	Lingen, Germany
JU1088	Kakegawa, Japan
JU1171	Concepcion, Chile

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