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DEPARTMENT OF BIOLOGY

Protective role of the *C. elegans* DBL-1/TGF-β signaling pathway in innate immune defenses against a panel of Gram-negative and Gram-positive bacteria

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Background

C. elegans exhibits different types of responses to immune challenges; the first response is avoidance of pathogens, which also shows the ability of animals to differentiate food sources, because these roundworms eat bacteria[1]. The second mechanism is protection through physical (exoskeleton) and mechanical means (pharyngeal grinding of bacteria)[2]. The third mechanism is induction of genes that include antimicrobial genes[2]. One of the signaling pathways involved in the innate immune response is the TGF-β pathway. DBL-1 is a ligand of a TGF-β signaling pathway in *C. elegans* known to regulate innate immunity genes[3,4]. Previous studies show DBL-1 regulates expression of innate immune response genes in response to Gram-negative *Serratia marcescens* Db11[5]. Is DBL-1 signaling required for an effective response to a range of bacterial pathogens or to a specific type of pathogen?

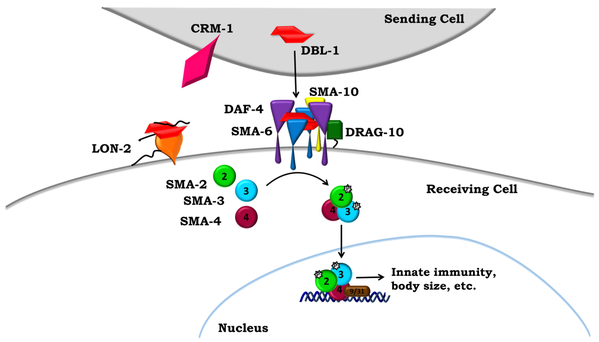


Figure 1. Canonical DBL-1/TGF-β signaling pathway in *C. elegans*. Adapted from Gumienny and Savage-Dunn (2005)[3].

Hypothesis

We propose that DBL-1 is required to mount an effective innate immune response to a variety of pathogenic organisms.

The panel of pathogenic bacteria to test our hypothesis includes:

Gram-positive bacteria	Gram-negative bacteria
Bacillus megaterium	*Enterobacter cloacae*
Enterococcus faecalis	*Klebsiella oxytoca*
Staphylococcus epidermidis	*Serratia marcescens*

Results

1. Do nematodes avoid varied pathogenic environments in a DBL-1-dependent manner?

A: Animals lacking DBL-1/TGF-β avoid Gram-negative but not Gram-positive bacteria

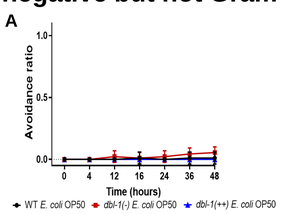
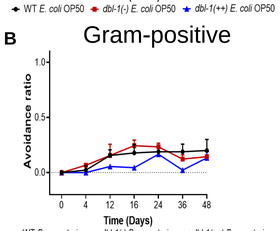
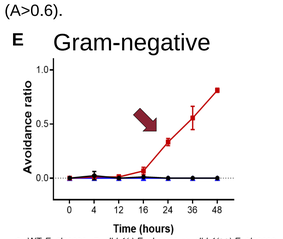
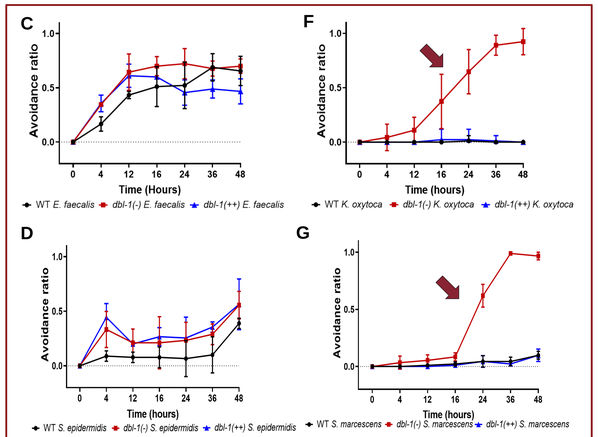


Figure 2. Avoidance behavior of three *dbl-1* genotypes to Gram-positive and -negative bacteria

Error bars represent standard deviation. Arrows mark strong aversion behavior differences (A>0.6).







- Wild-type N2 animals and *dbl-1* mutant animals were picked onto EZ plates spotted with test or control bacteria. Number of worms on the bacterial spot and off the bacterial spot at every time interval was recorded and aversion ratio was calculated (A= number of animals off the lawn/total number of animals). Assays were performed in triplicates and statistical analyses were performed by repeated measures ANOVA.

2. Does DBL-1 affect the feeding response of nematodes to pathogenic bacteria?

A: Reduced feeding by loss of DBL-1 and in response to Gram-positive bacteria is additive

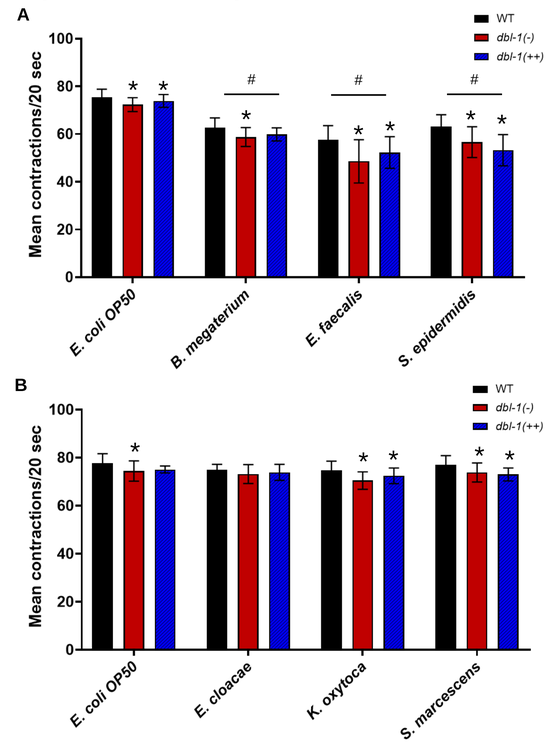


Figure 3. Pharyngeal pumping rate of the three *dbl-1* genotypes of *C. elegans* to an array of Gram-negative and -positive bacteria

E. coli OP50 is the control. Error bars represent standard deviation. *, p< 0.05 compared to the wild type on the same bacterial strain. #, p< 0.001 compared to the wild type on *E. coli*.

- Wild-type N2 animals and *dbl-1* mutants were picked onto EZ plates spotted with test or control bacteria. The number of pharyngeal contractions were counted for 20 seconds. Two counts were made for each animal and averaged. Assays were performed in triplicates and statistical analyses were performed by two-way ANOVA.

3. Does DBL-1 affect the lifespan of nematodes exposed to different pathogenic bacteria?

A: Animals lacking DBL-1/TGF-β have shorter lifespans on select pathogens

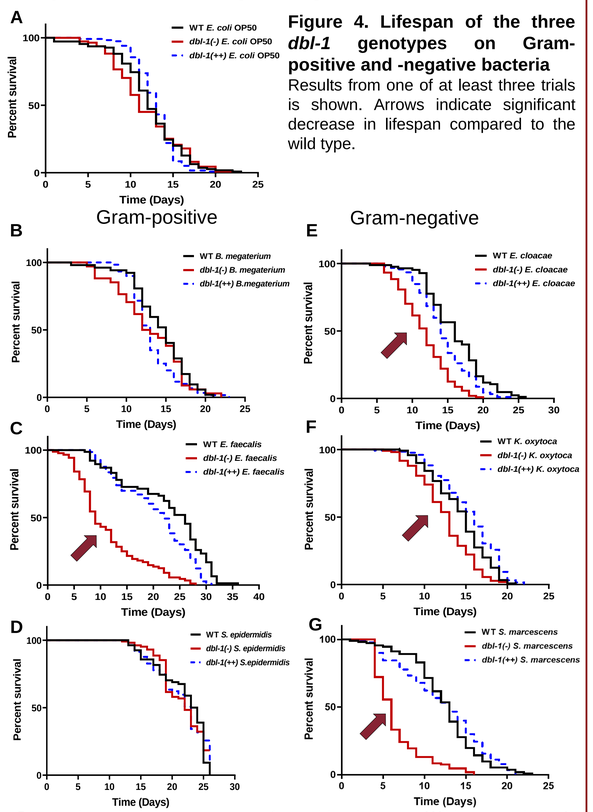


Figure 4. Lifespan of the three *dbl-1* genotypes on Gram-positive and -negative bacteria

Results from one of at least three trials is shown. Arrows indicate significant decrease in lifespan compared to the wild type.

- Standard survival assays were performed in triplicates. Wild-type and *dbl-1* mutant animals were picked onto EZ plates spotted with test or control bacteria. Results were analyzed using the log-rank test.

5. Does DBL-1 affect regulation of innate immunity genes?

A: DBL-1 mediates innate immune gene expression responses to Gram-positive and -negative bacteria.

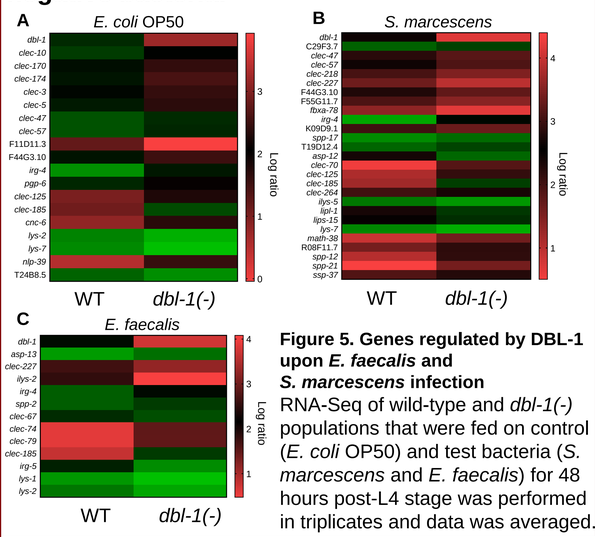


Figure 5. Genes regulated by DBL-1 upon *E. faecalis* and *S. marcescens* infection

RNA-Seq of wild-type and *dbl-1(-)* populations that were fed on control (*E. coli* OP50) and test bacteria (*S. marcescens* and *E. faecalis*) for 48 hours post-L4 stage was performed in triplicates and data was averaged.

4. Is a DBL-1 pathway reporter affected by pathogenic bacterial infection?

A: Select pathogens further increase reporter activity in *dbl-1(-)* animals

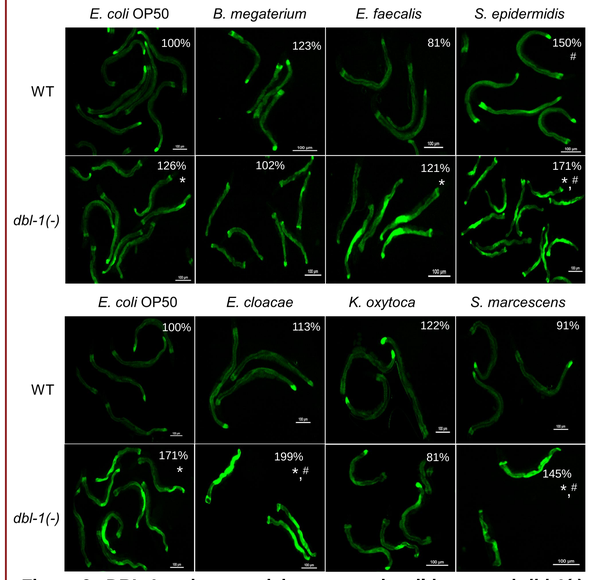


Figure 6. DBL-1 pathway activity reporter in wild-type and *dbl-1(-)* backgrounds fed on Gram-positive and -negative bacteria

- Animals expressing the green fluorescent protein reporter (*texIs127 [spp-9p::gfp]*) in wild-type and *dbl-1* mutant backgrounds were picked onto EZ plates spotted with test or control bacteria. *texIs127* is derived from *wkEx52* [6].Statistical analyses were performed by two-way ANOVA. Error bars represent standard deviation. *, p<0.05 compared to the wild type on the same bacteria. #, p<0.05 compared to the same worm strain on the *E. coli* OP50. Percent intensity is relative to respective worm strain on *E. coli* OP50.

Conclusions

Avoidance	Gram +: Avoidance response is independent of DBL-1 Gram -: Animals lacking DBL-1 display high avoidance
Survival	Gram +: Loss of DBL-1 makes nematodes more susceptible to select bacteria Gram -: Loss of DBL-1 makes nematodes more susceptible
Feeding	Gram +: Loss of DBL-1 and infection is additive Gram -: Loss of DBL-1 and *K. oxytoca* infection is additive
IIR genes	DBL-1 is required for induction of common and specific antimicrobial genes in response to Gram-positive and -negative bacteria
Reporter study	Exposure to some Gram-positive and -negative bacteria further up-regulates *spp-9* reporter expression

Future directions

- Validate RNA-seq results by real-time PCR.
- Characterize role of *spp-9* in the context of immunity.
- Determine role of DBL-1-mediated effects on surface coat (**1155B**) and cuticle (**447B**), which help protect animals from infection.

References

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Acknowledgements

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