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An Investigation of the Potential Antifungal Properties of CNC-2 in *Caenorhabditis elegans*

Angelina M. D. Zehrbach,[1] Alexandra R. Rogers,[2] and D. Ellen K. Tarr[2,3]

Abstract: Caenorhabditis elegans responds to infections by upregulating specific antimicrobial peptides. The caenacin-2 (*cnc-2*) gene is consistently upregulated in *C. elegans* by infection with the filamentous fungus *Drechmeria coniospora*, but there have been no direct studies of the CNC-2 peptide's in vivo or in vitro role in defending the nematode against this pathogen. We compared infection of wild-type and *cnc-2* knockout nematode strains with four potential pathogens: *D. coniospora*, *Candida albicans*, *Staphylococcus aureus*, and *Bacillus subtilis*. There was no significant difference in survival between strains for any of the pathogens or on the maintenance strain of *Escherichia coli*. While we were unable to demonstrate definitively that CNC-2 is integral to fungal defenses in *C. elegans*, we identified possible explanations for these results as well as future work that is needed to investigate CNC-2's potential as a new antifungal treatment.

Key words: antifungal peptide, caenacin, CNC-2, *Drechmeria coniospora*, host-parasite relationship.

Antimicrobial peptides (AMP) are the most important and numerous innate immune system effector molecules, with their antimicrobial activity usually due to disruption of the microbial membrane or cell wall (Bogaerts et al., 2010). Pathogens usually encounter constitutively expressed AMPs in addition to a specific mixture of induced AMPs upon host invasion (Bogaerts et al., 2010), reducing the likelihood that they will develop AMP resistance.

Caenorhabditis elegans is routinely used for studies of innate immunity and expresses three groups of AMPs: antibacterial factors (invertebrate defensins), caenopores (saposin-like proteins), and caenacins (a specific group of neuropeptide-like proteins) (Bogaerts et al., 2010; Tarr, 2012). While the cysteine-rich antibacterial factors and caenopores have homologs in many other nematode species, the glycine-rich caenacins seem to be more specific to *C. elegans* (Bogaerts et al., 2010; Tarr, 2012). Glycine-rich peptides are known for being able to inhibit the growth of fungi (Baumann et al., 2010; Herbinière et al., 2005; Sperstad et al., 2009).

Of 11 caenacins expressed by *C. elegans*, caenacin 2 (*cnc-2*) and the genes of the "*cnc-2* cluster" (*cnc-1*, *cnc-2*, *cnc-3*, *cnc-4*, *cnc5*, and *cnc-11*) have been the most investigated. The expression of *cnc-2* is upregulated in response to infection by the endoparasitic, nematophagous fungus *Drechmeria coniospora* (Zugasti and Ewbank, 2009). In addition, overexpression of genes in the "*cnc-2* cluster" (*cnc-1*, *cnc-2*, *cnc-3*, *cnc-4*, *cnc5*, and *cnc-11*) increases resistance to *D. coniospora* (Zugasti and Ewbank, 2009). This fungus infects *C. elegans* via conidial adhesion to the cuticle, penetration of the cuticle and epidermis, and hyphal growth inside the worm (Jansson et al., 1984; Gams and Jansson, 1985). Experiments using green fluorescent protein (GFP) and mCherry reporter constructs have confirmed that *cnc-2* expression is localized to the epidermis (Zugasti and Ewbank, 2009). The structure of this peptide, like most glycine-rich peptides, remains unknown (Baumann et al., 2010).

Thus far, *D. coniospora* is the only pathogen that consistently induces expression of *cnc-2*. By contrast, *Candida albicans* infects *C. elegans* via the intestinal lumen after ingestion and *cnc-2* has not been implicated in response to this infection (Pukkila-Worley et al., 2011). *Candida albicans* is the causative agent of candidiasis and the most common fungal infection in humans (Pukkila-Worley et al., 2009). In tests with bacterial pathogens, *cnc-2* is not upregulated in response to infection with *Microbacterium nematophilum* (O'Rourke et al., 2006), but it did increase in response to infection with *Staphylococcus aureus* (Bond et al., 2014).

The epidermal localization of *cnc-2* is consistent with its hypothesized activity against *D. coniospora* and a specific role in epidermal defense; however, there have been no in vitro or in vivo studies of the CNC-2 peptide in isolation to support this. We hypothesized that CNC-2 has antifungal activity against filamentous fungi, and we aimed to demonstrate this using in vivo assays. Specifically, we hypothesized that *C. elegans* lacking *cnc-2* will have an increased rate of mortality compared with wild-type *C. elegans* in the presence of the filamentous fungal pathogen *D. coniospora*, but not in the presence of *C. albicans* or bacterial pathogens. The goal of these studies was to verify the in vivo role of CNC-2 in the response to *D. coniospora*.

Materials and Methods

Nematode strains and maintenance: Wild-type (N2) and *cnc-2* knockout mutant (RB2374: *cnc-2* [ok3226] V) strains were provided by the Caenorhabditis Genetics Center, which is funded by the NIH Office of Research

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[1]Master of Biomedical Sciences Program, College of Health Sciences, Midwestern University, Glendale, AZ 85308.
[2]Department of Microbiology and Immunology, Arizona College of Osteopathic Medicine, Midwestern University, Glendale, AZ 85308.
[3]Current address: Division of Math and Sciences, Rock Valley College, Rockford, IL 61108.
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E-mail: e.tarr@rockvalleycollege.edu.
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Infrastructure Programs (P40 OD010440). The RB2374 strain was generated by the *C. elegans* Gene Knockout Project at Oklahoma Medical Research Foundation (OMRF), part of the International *C. elegans* Gene Knockout Consortium, using ethyl methanesulfate to knock out *cnc-2*. RB2374 was 8x backcrossed with N2 and renamed DET42 before initiation of the study. To confirm the deletion of *cnc-2*, the primer sequences suggested by the OMRF Knockout Group were used to amplify the region surrounding *cnc-2* from RB2374 genomic DNA. Outer left primer: 5′-ACCACTCCTTTGGTCTCGAA-3′; outer right primer: 5′-TCGACGTCATCATTTGGTTC-3′; inner left primer: 5′-TTTTGGAAGTCGACCGAAAC-3′; and inner right primer: 5′-CATATCAGTTGTGAGTATC-AATGGAA-3′. These primers were also used for sequencing by the College of Liberal Arts and Sciences DNA Laboratory at Arizona State University (Tempe, Arizona). The sequence of the *cnc-2* knockout mutant was compared with the corresponding region of cosmid R09B5 (GenBank accession number FO081061.1), and we confirmed a 509-bp deletion that included the complete *cnc-2* coding region. The sequence of the *cnc-2(ok3226)* allele was then deposited in WormBase (WormBase ID: WBVar00094290). Nematode strains were maintained on 35 mm Nematode Growth Media (NGM) agar plates supplemented with *E. coli* OP50 at 20°C. NGM plates were prepared as previously described except the final agar concentration was 1.8% (w/v) instead of 1.7% (w/v) (Brenner, 1974).

Fungal strains and maintenance: D. coniospora, ATCC® 18767™, was cocultured with wild-type (N2) *C. elegans* and maintained on 10 cm NGM plates at 20–25°C as previously described (Powell and Ausubel, 2008). *C. albicans*, ATCC® 64385™, was maintained on 10 cm Yeast-Peptone-Dextrose (YPD) agar plates at 25°C.

Bacterial strains and maintenance: S. aureus ATCC® 29213™ and *Bacillus subtilis* PY79 were grown at 37°C on Tryptic Soy Agar (TSA) plates. PY79 was provided by the Bacillus Genetic Stock Center (Columbus, OH).

Caenorhabditis elegans *lifespan assay:* Wild-type and *cnc-2* knockout L4 worms were transferred to 35 mm NGM + *E. coli* OP50 plates (20 worms per plate, 5 plates per strain) and incubated at either 20 or 25°C. Assays were performed at both 20 and 25°C because while the worms are routinely maintained at 20°C, 25°C is closer to the optimum temperature for the pathogens used in these studies. Worms were scored daily as alive, dead, or censored until there were no longer surviving worms. Death was defined as absence of any movement, even upon nudging by the end of a worm pick (platinum wire). A worm was censored when not found on the plate (usually because it had crawled up the side and desiccated) or scored as censored for the following day if death occurred during transfer to a new plate. Worms were transferred to fresh plates on a daily basis until they stopped laying eggs to prevent the progeny from affecting the scoring.

Caenorhabditis elegans *brood size assay:* Wild-type and *cnc-2* knockout L4s were transferred to 35 mm NGM + *E. coli* OP50 plates (1 worm per plate and 25 plates per strain) and incubated at either 20 or 25°C. Worms were allowed to mature and lay all eggs that they carried. For convenience, they were transferred daily to a fresh plate so that the prior plate's total progeny could be counted. The progeny were tallied after having matured to the L1 or L2 stage to allow for easier counting and to make sure only viable offspring were being recorded.

Drechmeria coniospora *pathogenicity assay:* Wild-type and *cnc-2* knockout L4 worms were transferred to 35 mm NGM + *E. coli* OP50 plates (20 worms per plate, 5 plates per strain), innoculated with freshly harvested *D. coniospora* spore solution (1×10^7 spores per plate) and incubated at 25 or 15°C. Worms were marked as alive, dead, or censored every 24 hr for 5 d (25°C) or 6 d (15°C), with any remaining live worms censored on day 6 (25°C) or 7 (15°C). Live worms were transferred daily to fresh assay plates to prevent progeny from affecting scoring.

Candida albicans *pathogenicity assay: C. albicans* was grown at 30°C overnight on Brain Heart Infusion (BHI) agar (BD Ref. #237500) supplemented with 45 μg/mL kanamycin (KAN). The next day, a single colony was inoculated into 1 mL of YPD broth and grown overnight at 30°C with agitation. Ten microliters of this culture was spread onto BHI agar plates supplemented with 45 μg/mL KAN and incubated for approximately 20 hr at 30°C. After incubation, excess yeast was scraped off the agar plates using a sterile loop or glass spreader to aid in the eventual visual scoring of worms. The agar surface was left intact. Wild-type and *cnc-2* knockout L4s were then added to the plates (20 worms per plate, 5 plates per strain) and incubated at 25°C. Worms were scored as alive, dead, or censored each day for 5 d, with any remaining live worms censored on day 6. Live worms were transferred daily to fresh assay plates to prevent progeny from affecting scoring.

Staphylococcus aureus *pathogenicity assay: S. aureus* was grown at 37°C overnight on TSA. The next day, a single colony was inoculated into 3 mL of Tryptic Soy Broth (TSB) and grown overnight at 37°C with agitation. Ten microliters of a 1:10 dilution of this culture was spread onto TSA plates supplemented with 5 μg/mL nalidixic and incubated for 3–6 hr at 37°C. Wild-type and *cnc-2* knockout L4s were then added to the plates (20 worms per plate and 5 plates per strain) and incubated at 25°C. Worms were scored as alive, dead, or censored each day for 5 d, with any remaining live worms censored on day 6. Live worms were transferred daily to fresh assay plates to prevent progeny from affecting scoring.

Bacillus subtilis *pathogenicity assay: B. subtilis* was grown at 37°C overnight on TSA. The next day, a single colony was inoculated into 3 mL of TSB and grown overnight at 37°C with agitation. Ten microliters of this culture was spread onto TSA plates and incubated

for 3–6 hr at 37°C. Wild-type and *cnc-2* knockout L4s were then added to the plates (20 worms per plate, 5 plates per strain) and incubated at 25°C. Worms were scored as alive, dead, or censored each day for 5 d, with any remaining live worms censored on day 6. Live worms were transferred daily to fresh assay plates to prevent progeny from affecting scoring.

Statistics: Kaplan-Meier survival curves were generated from the lifespan and pathogenicity assays using GraphPad Prism software. These curves were then compared using the logrank test. Differences in brood size between strains were evaluated using an unpaired *t*-test. For all assays, a value of $P < 0.05$ indicated a significant difference and error bars represented the SE.

Results

Overall fitness of wild-type and cnc-2 *knockout strains:* There were no significant differences between the lifespans of the wild-type and *cnc-2* knockout strains at 20 or 25°C (Fig. 1) when grown on standard NGM plates with *E. coli* OP50 as the food source. Similarly, there were no significant differences in the brood size at either 20 or 25°C (Fig. 2).

Pathogen susceptibility in vivo: To assess the contribution of CNC-2 to the fungal defenses of *C. elegans*, we compared the susceptibility of the wild-type and *cnc-2* knockout strains in vivo to pathogenic fungi. We observed no significant difference in survival curves between the wild-type and *cnc-2* knockout strains of *C. elegans* when exposed to the filamentous fungus *D. coniospora* or the yeast *C. albicans* (Fig. 3). Similarly, there was no significant difference in survival between the two strains when exposed to *S. aureus* ATCC 29213 or *B. subtilis* PY79 (Fig. 4).

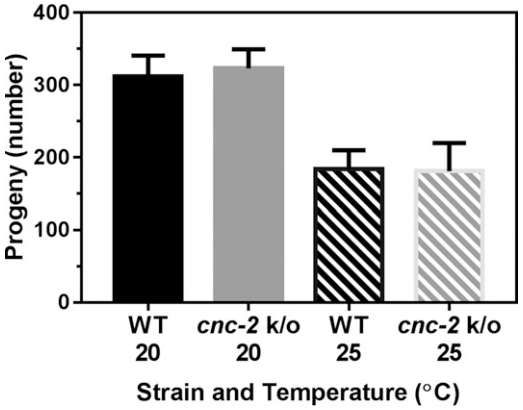

Fig. 2. Average brood size of wild-type (WT) and *cnc-2* knockout (k/o) *Caenorhabditis elegans* at 20 and 25°C. The number of progeny produced by individual wild-type (black bar, black and white striped bar) and *cnc-2* knockout (grey bar and grey- and white-striped bar) worms was counted at 20°C (filled bars) and 25°C (striped bars). Bars represent the means and standard deviations of 100 worms per condition. There was no significant difference between wild-type and *cnc-2* knockout strains at either temperature: $P = 0.1424$ (20°C) and $P = 0.8064$ (25°C).

Discussion

The lifespan and brood size assays established that knocking out *cnc-2* did not affect the overall fitness of *C. elegans* and that our experimental knockout strain was as healthy as wild-type before its exposure to potential pathogens.

Previous work showed that *C. elegans* has a survival time of less than 3 d when exposed to *D. coniospora* at 25°C and less than 5 d at 15°C (Couillault et al., 2004; Pujol et al., 2008; Zugasti and Ewbank, 2009). The strain usually used in these assays is NY [5] (ATCC

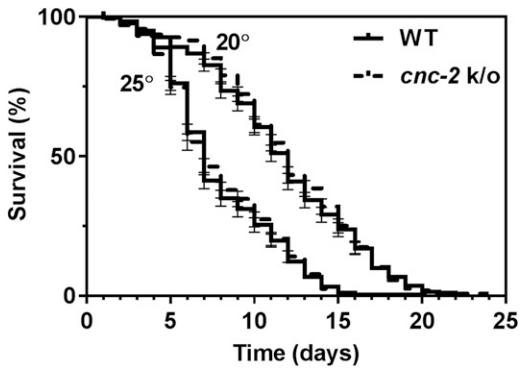

Fig. 1. Average lifespan of wild-type (WT) and *cnc-2* knockout (k/o) *Caenorhabditis elegans* at 20 and 25°C. Wild-type (solid line) and *cnc-2* knockout (dashed line) worms were fed standard *Escherichia coli* OP50 and scored every day as alive, dead, or censored until no worms survived. Worms were transferred to fresh plates for the first several days to avoid confusion with progeny. Results are shown as the means of three independent experiments (error bars show standard error); each experiment included 100 worms for each strain. There was no significant difference between wild-type and *cnc-2* knockout strains at either temperature: $P = 0.5423$ (20°C) and $P = 0.8089$ (25°C).

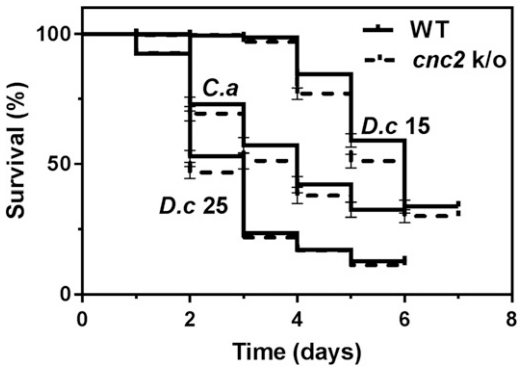

Fig. 3. Susceptibility of wild-type (WT) and *cnc-2* knockout (k/o) worms to the fungi *Candida albicans* and *Drechmeria coniospora*. Wild-type (solid line) and *cnc-2* knockout (dashed line) worms were grown in the presence of *C. albicans* (*C.a*) or *D. coniospora* (*D.c*) and scored every day as alive, dead, or censored for five (*C. albicans* and *D. coniospora* at 25°C) or 6 d (*D. coniospora* at 15°C). Experiments with *D. coniospora* also included *Esherichia coli* OP50 as a food source. Results are shown as means and standard errors for independent experiments using 100 worms of each strain; none of the differences were statistically significant: $n = 3$ for *C. albicans* ($P = 0.5997$), $n = 5$ for *D. coniospora* at 25°C ($P = 0.2017$), and $n = 4$ for *D. coniospora* at 15°C ($P = 0.0582$).

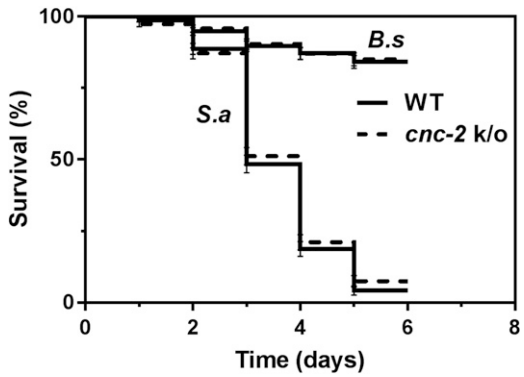

FIG. 4. Susceptibility of wild-type (WT) and *cnc-2* knockout (k/o) worms to the Gram + bacteria *Bacillus subtilis* and *Staphylococcus aureus*. Wild-type (solid line) and *cnc-2* knockout (dashed line) worms were grown in the presence of *B. subtilis* (*B.s*) or *S. aureus* (*S.a*) and scored every day as alive, dead, or censored for 5 d. Results are shown as the means of three independent experiments (error bars show standard error); each experiment included 100 worms for each strain. There was no significant difference between wild-type and *cnc-2* knockout strains for either bacterial species: $P = 0.7230$ (*B. subtilis*) and $P = 0.4764$ (*S. aureus*).

96282), but this strain showed limited infectivity of *C. elegans* in our hands. We achieved similar levels of infectivity to those previously reported by using strain 10771 (ATCC 18767). Although CNC-2 is expected to play a role in the response to *D. coniospora* infection, wild-type worms are susceptible to infection in spite of CNC-2's presence. Because transgenic worms overexpressing the *cnc-2* cluster took longer to succumb to infection with *D. coniospora* (Zugasti and Ewbank, 2009), it seemed possible the *cnc-2* knockout would show a shorter course of infection than wild-type. Although there were fewer *cnc-2* knockout worms surviving at most time points of the *D. coniospora* assays (Fig. 3), the difference did not reach a statistical significance at either 25°C ($P = 0.2017$) or 15°C ($P = 0.0582$). This might be explained by some functional redundancy between *cnc-2* and other genes in the *cnc-2* cluster: because the worms in this study were only lacking *cnc-2*, it is possible that other genes in the cluster compensate for its function in immunity against *D. coniospora*. It is also possible that because the presence of CNC-2 does not render the worms resistant to *D. coniospora* infection, further susceptibility resulting from *cnc-2* deletion may be difficult to detect. Further testing with filamentous fungi that are not pathogenic to wild-type worms may eventually reveal the extent of CNC-2's ability to protect against fungal infection.

Both wild-type and *cnc-2* knockout *C. elegans* strains showed approximately 25% survival at day 6 of exposure to the yeast *C. albicans* (Fig. 3), which is consistent with previous reports for wild-type susceptibility to *C. albicans* (Breger et al., 2007; Pukkila-Worley et al., 2011). CNC-2 has not been implicated in the response to *C. albicans*, which usually infects *C. elegans* via the intestine, where *cnc-2* is not expressed. However, previous results did not rule out the possibility that CNC-2 effectively protects the epidermis from infection under normal circumstances and loss of CNC-2 at this surface might remove this barrier to infection.

CNC-2 is generally considered to be an antifungal peptide, although a recent study did show an increase in *cnc-2* expression in response to infection with *S. aureus* (Bond et al., 2014). There was no significant difference between wild-type and *cnc-2* knockout when exposed to *S. aureus* (Fig. 4), although it is possible that because *S. aureus* is highly pathogenic to *C. elegans*, the course of infection is already as short as possible. The study of immune deficiencies can reveal the functions of immune molecules in basic immune responses that are generally obscured with an intact immune system. To confirm that CNC-2 was unlikely to have a previously unidentified role in the general response to Gram-positive bacteria, we also exposed the worms to the nonpathogenic PY79 strain of *B. subtilis*. Again, there was no difference between the strains (Fig. 4).

Effective antifungal drugs are in high demand because of drug resistance, toxicity, and a rise in infection rates, so that makes research that strives toward coming up with new ones very important (Sangamwar et al., 2008; Pukkila-Worley et al., 2009). Endemic to the southwestern United States, coccidiomycosis has become an increasing cause of hospital stays, up from 2.3 initial hospitalizations/100,000 population in 2000 to 5.0/100,000 in 2011, at a cost of more than 2 billion dollars during this time period (Sondermeyer et al., 2013). It has been estimated that the cost of nosocomial candidemia approaches 1 billion dollars for the United States annually (Miller et al., 2001; Breger et al., 2007). High-throughput assays that can be used to develop novel drugs are very desirable and because the genome of *C. elegans* has been completely described, an assay that can combine *C. elegans* in a high-throughput assay would be well received (Pukkila-Worley et al., 2009). Research into AMP shows promise for the development of new antifungals. However, there is also an increasing acknowledgment that their immunomodulatory effects may be at least as important as their direct antimicrobial activity (Hancock et al., 2016). Although some direct activity has been shown for the related peptide, NLP-31 (Couillault et al., 2004), we have thus far been unsuccessful in confirming the activity of recombinant CNC-2 in vitro. Further work is needed to determine whether or not CNC-2 has potential as an antifungal agent.

LITERATURE CITED

Baumann, T., Kämpfer, U., Schürch, S., Schaller, J., Largiadèr, C., Nentwig, W., and Kuhn-Nentwig, L. 2010. Ctenidins: Antimicrobial glycine-rich peptides from hemocytes of the spider *Cupiennius salei*. Cellular and Molecular Life Sciences 67:2787–2798.

Bogaerts, A., Beets, I., Schoofs, L., and Verleyen, P. 2010. Antimicrobial peptides in *Caenorhabditis elegans*. Invertebrate Survival Journal 7:45–52.

Bond, M. R., Ghosh, S. K., Wang, P., and Hanover, J. A. 2014. Conserved nutrient sensor O-GlcNAc transferase is integral to *C. elegans* pathogen-specific immunity. PLoS ONE 9:e113231.

Breger, J., Fuchs, B. B., Aperis, G., Moy, T. I., Ausubel, F. M., and Mylonakis, E. 2007. Antifungal chemical compounds identified using a *C. elegans* pathogenicity assay. PLoS Pathogens 3:e18.

Brenner, S. 1974. The genetics of *Caenorhabditis elegans*. Genetics 77:71–94.

Couillault, C., Pujol, N., Reboul, J., Sabatier, L., Guichou, J.-F., Kohara, Y., and Ewbank, J. J. 2004. TLR-independent control of innate immunity in *Caenorhabditis elegans* by the TIR domain adaptor protein TIR-1, an ortholog of human SARM. Nature Immunology 5:488–494.

Gams, W., and Jansson, H.-B. 1985. The nematode parasite Meria coniospora Drechsler in pure culture and its classification. Mycotaxon 22:33–38.

Hancock, R. E. W., Haney, E. F., and Gill, E. E. 2016. The immunology of host defence peptides: Beyond antimicrobial activity. Nature Reviews Immunology 16:321–334.

Herbinière, J., Braquart-Varnier, C., Grève, P., Strub, J.-M., Frère, J., Van Dorsselaer, A., and Martin, G. 2005. Armadillidin: A novel glycine-rich antibacterial peptide directed against gram-positive bacteria in the woodlouse *Armadillidium vulgare* (Terrestrial Isopod, Crustacean). Developmental and Comparative Immunology 29:489–499.

Jansson, H.-B., von Hofsten, A., and von Mecklenburg, C. 1984. Life cycle of the endoparasitic nematophagous fungus *Meria coniospora*: A light and electron microscopic study. Antonie van Leeiwenhoek 50:321–327.

Miller, L. G., Hajjeh, R. A., and Edwards, J. E., Jr. 2001. Estimating the cost of nosocomial candidemia in the United States. Clinicla Infectious Diseases 32:1110.

O'Rourke, D., Baban, D., Demidova, M., Mott, R., and Hodgkin, J. 2006. Genomic clusters, putative pathogen recognition molecules, and antimicrobial genes are induced by infecton of *C. elegans* with *M. nematophilum*. Genome Research 16:1005–1016.

Powell, J. R., and Ausubel, F. M. 2008. Models of *Caenorhabditis elegans* infection by bacterial and fungal pathogens. Methods in Molecular Biology 415:403–427.

Pujol, N., Zugasti, O., Wong, D., Couillault, C., Kurz, C. L., Schulenburg, H., and Ewbank, J. J. 2008. Anti-fungal innate immunity in *C. elegans* is enhanced by evolutionary diversification of antimicrobial peptides. PLoS Pathogens 4:e1000105.

Pukkila-Worley, R., Ausubel, F. M., and Mylonakis, E. 2011. Candida albicans infection of *Caenorhabditis elegans* induces antifungal immune defenses. PLoS Pathogens 7:e1002074.

Pukkila-Worley, R., Holson, E., Wagner, F., and Mylonakis, E. 2009. Antifungal drug discovery through the study of invertebrate model hosts. Current Medicinal Chemistry 16:1588–1595.

Sangamwar, A. T., Deshpande, U. D., and Pekamwar, S. S. 2008. Antifungals: Need to search for a new molecular target. Indian Journal of Pharmaceutical Sciences 70:423–430.

Sondermeyer, G., Lee, L., Gilliss, D., Tabnak, F., and Vugia, D. 2013. Coccidioidomycosis-associated hospitalizations, California, USA, 2000–2011. Emerg Infect Dis 19:1590–1597.

Sperstad, S. V., Haug, T., Vasskog, T., and Stensvåg, K. 2009. Hyastatin, a glycine-rich multi-domain antimicrobial peptdie isolated from the spider crab (*Hyas araneus*) hemocytes. Molecular Immunology 46:2604–2612.

Tarr, D. E. K. 2012. Nematode antimicrobial peptides. Invertebrate Survival Journal 9:122–133.

Zugasti, O., and Ewbank, J. J. 2009. Neuroimmune regulation of antimicrobial peptide expression by a noncanonical TGF-beta signaling pathway in *Caenorhabditis elegans* epidermis. Nature Immunology 10:249–256.