International Journal of Biomedical And Advance Research

ISSN: 2229-3809 (Online
Journal DOI:10.7439/ijbar
CODEN:IJBABN

Review Article

Recent advances in *C. Elegans* as a model system for high throughput antimicrobial drug discovery

Raj Mohan Raja Muthiah*

Massachusetts General Hospital, Boston, Massachusetts, United States

***Correspondence Info:**
Raj Mohan Raja Muthiah,
Massachusetts General Hospital,
Boston, Massachusetts, United States
E -mail: rrajamuthiah@partners.org

Abstract

Multidrug resistance among pathogens has become a leading cause of health care concern since these pathogens have acquired resistance against most classes of antimicrobial drugs. Therefore there is urgent need for newer antimicrobial compounds and new treatment strategies. Unfortunately the pace of new antimicrobial drug discovery programs have slowed down due to rising cost of drug discovery coupled with the relatively unattractive markets for antimicrobial drugs compared to drugs for chronic diseases. In this background, invertebrate model organisms such as the nematode *Caenorhabditis elegans* and fruit fly *Drosophila melanogaster* are finding increasing use as models to study host-pathogen relationship and high throughput antimicrobial drug discovery. The focus of this review will be to give a brief outline of the *C. elegans* model for high throughput antimicrobial drug discovery.

Keywords: *Caenorhabditis elegans*, infectious diseases, drug resistance, high throughput screening, drug discovery.

1. Introduction

In the past several decades, antimicrobial drugs have played an important role in the overall improvement in human lifespan. In veterinary medicine, antimicrobial drugs have led to significant improvements in the quality of animal products. However, the positive effects of these drugs have been negated by their rampant use/misuse. The incidence of "antimicrobial resistance" or non-susceptibility of microorganisms to drugs that they were previously susceptible to is rapidly increasing[1,2]. This phenomenon of antimicrobial resistance needs to be countered with social education on responsible antimicrobial use and also with robust antimicrobial drug discovery programs.

Traditional methods of antimicrobial drug discovery involve *in vitro* screening and selection of test compounds, optimizing compound structure by performing Structure Activity Relationships (SAR analysis and lastly testing the compound in an in vivo disease model[3,4]. The process of transforming the compound identified in the lab into a drug that is safe for human use is both long and expensive taking up to 15 years and costing as much as a billion dollars. Additionally, the therapeutic window of antimicrobial use is relatively short due to rapid emergence of drug resistant strains. These reasons have led to mass exodus of antimicrobial discovery programs in big pharma, turning an already bad situation worse. In recent years, academic labs and small start-ups have been at the forefront of antimicrobial drug discovery.

The free living nematode *C. elegans* has recently become a popular model organism for studying pathogenesis of many bacterial and fungal pathogens[5]. Key virulence factors that are involved in pathogenesis in humans are also involved in pathogenesis in the nematodes. The signalling pathways by which *C. elegans*counters pathogens are also strikingly similar to those in metazoans[6,7]. *C. elegans* have a relatively short lifespan, are relatively inexpensive to maintain and using them as models to study host pathogen relationship does not raise the ethical or logistical concerns that arise while working with higher model organisms such as rodents. Moreover, using *C. elegans* as a test platform allows for simultaneous assessment of both the antimicrobial efficacy of the test compound and the compound's toxicity since if the compound is toxic the worms will still not survive even if cured of the infection. Often times, compounds that display a lot of potential based on in vitro antimicrobial assays, end up being too toxic when tested in vivo in animals.

Table 1: Lists the studies which used *C. elegans* for high throughput or semi-high throughput antimicrobial drug discovery and testing.

Pathogen	Reference
Bacteria:	
Enterococcus faecalis	8, 9
Pseudomonas aeruginosa	10
Staphylococcus aureus	11, 12
Fungi:	
Candida albicans	13, 14

2. Work flow of the *C. elegans* antimicrobial drug discovery platform

A typical work flow of the high throughput screening platform involving *C. elegans* is similar to as shown in Fig. 1. [11]. The strength of using *C. elegans* in a high throughput platform is that several tasks in the work flow can be automated, reducing the workload and at the same time improving experiment reliability. The *C. elegans glp-4(bn2;sek-1(km4* double mutant strain was used in several high throughput screening studies. The *glp-4(bn2* mutation renders the strain incapable of producing progeny at 25°C [15] and the *sek-1(km4* mutation enhances sensitivity to various pathogens[16], reducing assay time. Therefore, when the *glp-4;sek-1* strain is used, the worms can be infected with the pathogen and maintained at a temperature of 25°C which would be suitable for the growth of the pathogens. Moreover, exposure of the mutant worms to 25°C renders them sterile and therefore the assay outcome will not be complicated by the production of progeny worms.

C. elegans is normally maintained in the laboratory at 15°C with non-pathogenic *E. coli* as food source. After hatching from the egg, nematodes undergo four larval development stages (L1-L4 before reaching adult stage. While performing *C. elegans* infection assays, worms in their L4 stage are usually preferred since they are voracious eaters and would therefore ingest even pathogens fed to them as food. A

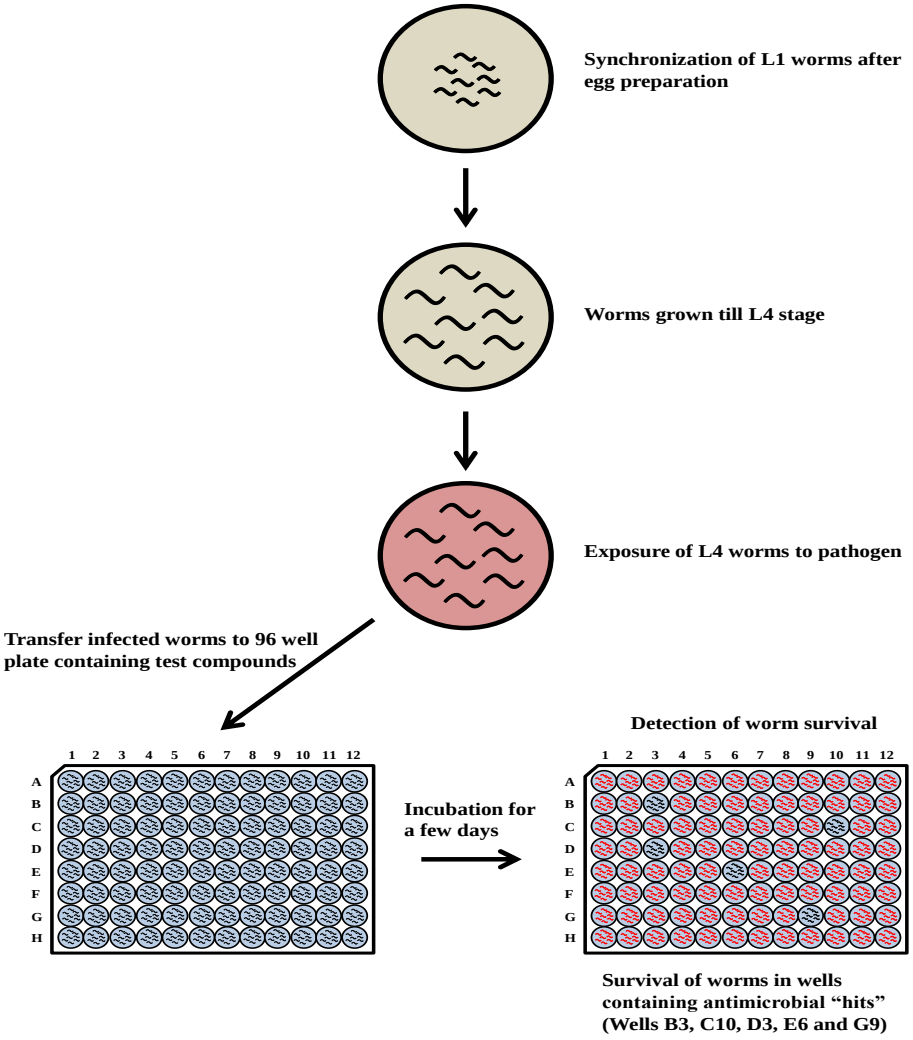
synchronized population of L4 worms can be obtained by following established protocols[11,17,18]. Briefly, the L1 stage worms are grown at 15°C on SK agar plates with *E. coli*HB101 strain as the food source for four days until the worms reach the gravid adult stage. Embryos are harvested from adult worms and the eggs are hatched by incubation in M9 buffer at 15°C for two days. Approximately 4,500 L1 hatchlings are then transferred to SK agar plates seeded with HB101. After incubation for 52 hours at the restrictive temperature of 25°C the worms would develop to sterile L4 or young adult stage. The worms are harvested by gently washing them off the plates with M9 buffer and are ready to be infected with the pathogen.

Infection is usually carried out by transferring the worms on to SK agar plates seeded with an overnight culture of the pathogen. After a period of incubation with the pathogen, the worms are washed off the plates and then added to 96 or 384 well plates containing test compounds using an automated large particle sorter such as Union Biometrica Complex Object Parametric Analyzer and Sorter (COPASBioSort). This approach might not be suitable for pathogens that form biofilm since biofilm producing pathogens adhere well to plastic surfaces such as tubings and might pose a lot of difficulty to remove them. Under these circumstances, it might be more suitable to co-infect the worms with the pathogen directly in the assay plate containing test compounds. Alternatively, the worms could also be manually introduced into the assay plate without using the sorter, which is not only laborious but also poses the risk of variability in the number of worms added to each well.

Worms are exposed to the test compounds in the assay plate over a period of 4-5 days which alloys sufficient time for the compound to manifest its antibacterial effect both directly on the bacteria and indirectly by modulating the worm's innate immune system. The beneficial effect of the compounds will have an effect of prolonging worm survival during the incubation period. Moreover, if the test compounds are toxic, it would also affect worm survival. At the end of the incubation period, worm survival can be detected by manually probing with a platinum pick, live worms will respond by withdrawing from the pick whereas dead worms will remain stationary. Worm survival measurement can also be automated by employing viability dyes such as propidium iodide or sytox orange and capturing worm fluorescence using Image Xpress Micro automated microscope (Molecular Devices. In this method, live worms will exclude the dye whereas dead worms will take up the dye and fluoresce. The percent fluorescence can then be calculated which will correspond to the death of worms in each well. With respect to fungal pathogens such as *Candida albicans*, high resolution bright field images are captured for each well to detect hyphae formation from fungal cells ingested by the worms. However, lack of hyphae formation does not necessarily indicate worm survival as *Candida* has been shown to kill *C. elegans* even without forming hyphae. Therefore, the worms in the wells where hyphae are not observed should still be tested for survival.

In order to identify the hit compounds, the Z score is calculated from the ratio X = (number of dead worms in the well/ (total number of worms in the well. The Z score is defined by the equation $Z = (X-\mu/\sigma$ where X is the raw sample score, μ is the mean score for all the wells and σ is the standard deviation of the wells in the assay plate. Samples having a Z score greater than 3σ are generally considered as hits.

Figure 1: Work flow of a typical antimicrobial high throughput screening platform using *C. elegans* as a whole animal host. Survival of the worms in a well containing test compound indicates that the compound is an antimicrobial hit.



3. Conclusion

The rising incidence of antimicrobial resistance among pathogens underscores the importance of identifying new treatment strategies and new methods for drug discovery. The *C. elegans* model for high throughput antimicrobial screening will not completely eliminate the necessity of testing potential leads in higher model system. Rather, it would serve to focus our efforts on the most promising hits by eliminating compounds which have no antimicrobial activity or too toxic, at a very early stage in the drug discovery and screening process.

References

1. Canton R, Bryan J. Global antimicrobial resistance: from surveillance to stewardship. Part 1: surveillance and risk factors for resistance. *Expert review of anti-infective therapy*. 2012 Nov;10(11:1269-71. PubMed PMID: 23241182.
2. Bell BG, Schellevis F, Stobberingh E, Goossens H, Pringle M. A systematic review and meta-analysis of the effects of antibiotic consumption on antibiotic resistance. BMC infectious diseases. 2014;14:13. PubMed PMID: 24405683. Pubmed Central PMCID: 3897982.
3. Silver LL. Challenges of antibacterial discovery. *Clinical microbiology reviews*. 2011 Jan;24(1:71-109. PubMed PMID: 21233508. Pubmed Central PMCID: 3021209.
4. Singh SB. Confronting the challenges of discovery of novel antibacterial agents. *Bioorganic & medicinal chemistry letters*. 2014 Aug 15;24(16:3683-9. PubMed PMID: 25017034.
5. Marsh EK, May RC. Caenorhabditis elegans, a model organism for investigating immunity. *Applied and environmental microbiology*. 2012 Apr;78(7:2075-81. PubMed PMID: 22286994. Pubmed Central PMCID: 3302602.
6. Zhang R, Hou A. Host-Microbe Interactions in Caenorhabditis elegans. *ISRN microbiology*. 2013;2013:356451. PubMed PMID: 23984180. Pubmed Central PMCID: 3747393.
7. Rajamuthiah R, Mylonakis E. Effector triggered immunity: Activation of innate immunity in metazoans by bacterial effectors. *Virulence*. 2014 May 6;5(7. PubMed PMID: 24802615. Pubmed Central PMCID: 4189875.
8. Moy TI, Ball AR, Anklesaria Z, Casadei G, Lewis K, Ausubel FM. Identification of novel antimicrobials using a live-animal infection model. Proceedings of the National Academy of Sciences of the United States of America. 2006 Jul 5;103(27:10414-9. PubMed PMID: 16801562. Pubmed Central PMCID: 1482800.
9. Moy TI, Conery AL, Larkins-Ford J, Wu G, Mazitschek R, Casadei G, et al. High-throughput screen for novel antimicrobials using a whole animal infection model. *ACS chemical biology*. 2009 Jul 17;4(7:527-33. PubMed PMID: 19572548. Pubmed Central PMCID: 2745594.
10. Zhou YM, Shao L, Li JA, Han LZ, Cai WJ, Zhu CB, et al. An efficient and novel screening model for assessing the bioactivity of extracts against multidrug-resistant Pseudomonas aeruginosa using Caenorhabditis elegans. *Bioscience, biotechnology, and biochemistry*. 2011;75(9:1746-51. PubMed PMID: 21897025.
11. Rajamuthiah R, Fuchs BB, Jayamani E, Kim Y, Larkins-Ford J, Conery A, et al. Whole animal automated platform for drug discovery against multi-drug resistant Staphylococcus aureus. *PloS one*. 2014;9(2:e89189. PubMed PMID: 24586584. Pubmed Central PMCID: 3929655.
12. Dolla NK, Chen C, Larkins-Ford J, Rajamuthiah R, Jagadeesan S, Conery AL, et al. On the Mechanism of Berberine–INF55 (5-Nitro-2-phenylindole Hybrid Antibacterials. *Australian Journal of Chemistry*. 2014 September 22;67(10:1471-80.
13. Breger J, Fuchs BB, Aperis G, Moy TI, Ausubel FM, Mylonakis E. Antifungal chemical compounds identified using a C. elegans pathogenicity assay. *PLoS pathogens*. 2007 Feb;3(2:e18. PubMed PMID: 17274686. Pubmed Central PMCID: 1790726.
14. Okoli I, Coleman JJ, Tampakakis E, An WF, Holson E, Wagner F, et al. Identification of antifungal compounds active against Candida albicans using an improved high-throughput Caenorhabditis elegans assay. PloS one. 2009;4(9:e7025. PubMed PMID: 19750012. Pubmed Central PMCID: 2737148.
15. Beanan MJ, Strome S. Characterization of a germ-line proliferation mutation in *C. elegans*. Development. 1992 Nov;116(3:755-66. PubMed PMID: 1289064. Epub 1992/11/01. eng.
16. Tanaka-Hino M, Sagasti A, Hisamoto N, Kawasaki M, Nakano S, Ninomiya-Tsuji J, et al. SEK-1 MAPKK mediates Ca2+ signaling to determine neuronal asymmetric development in Caenorhabditis elegans. *EMBO reports*. 2002 Jan;3(1:56-62. PubMed PMID: 11751572. Pubmed Central PMCID: 1083920.
17. Powell JR, Ausubel FM. Models of Caenorhabditis elegans infection by bacterial and fungal pathogens. *Methods in molecular biology*. 2008;415:403-27. PubMed PMID: 18370168.
18. Desalermos A, Tan X, Rajamuthiah R, Arvanitis M, Wang Y, Li D, et al. A Multi-Host Approach for the Systematic Analysis of Virulence Factors in Cryptococcus neoformans. *The Journal of infectious diseases*. 2014 Aug 11. PubMed PMID: 25114160.