Developing Galleria mellonella as a model host for human pathogens

Simon M. Cook  
*University of Wollongong*, scook@uow.edu.au

Jason D. McArthur  
*University of Wollongong*, jasonm@uow.edu.au

Follow this and additional works at: [https://ro.uow.edu.au/smhpapers](https://ro.uow.edu.au/smhpapers)

Part of the Medicine and Health Sciences Commons, and the Social and Behavioral Sciences Commons

**Recommended Citation**

Cook, Simon M. and McArthur, Jason D., "Developing Galleria mellonella as a model host for human pathogens" (2013). Faculty of Science, Medicine and Health - Papers: part A. 1011.  

Research Online is the open access institutional repository for the University of Wollongong. For further information contact the UOW Library: research-pubs@uow.edu.au
Developing Galleria mellonella as a model host for human pathogens

Abstract
The larvae of Galleria mellonella (also known colloquially as the wax worm) is increasingly being used as an infection model to study virulence factors and pathogenesis of many prominent bacterial and fungal human pathogens. When compared with traditional mammalian model hosts, invertebrate infection models are cheaper to establish and maintain, are more amenable to high-throughput studies and are not subjected to the same ethical constraints as vertebrates. In addition to these benefits, G. mellonella larvae possess a number of other characteristics which make these organisms particularly useful for the study of human pathogens. Larvae are relatively large in size (12-20 mm) which enables easy manipulation and facilitates the collection of tissue/ hemolymph samples for downstream analysis.

Keywords
developing, mellonella, galleria, model, host, human, pathogens, CMMB

Disciplines
Medicine and Health Sciences | Social and Behavioral Sciences

Publication Details

This journal article is available at Research Online: https://ro.uow.edu.au/smhpapers/1011
Developing *Galleria mellonella* as a model host for human pathogens

Simon M Cook and Jason D McArthur*

Illawarra Health and Medical Research Institute; School of Biological Sciences; University of Wollongong; Wollongong, NSW Australia

**Keywords:** model organism, infection, *Galleria mellonella*, immunity, pathogenesis

The larvae of *Galleria mellonella* (also known colloquially as the wax worm) is increasingly being used as an infection model to study virulence factors and pathogenesis of many prominent bacterial and fungal human pathogens. When compared with traditional mammalian model hosts, invertebrate infection models are cheaper to establish and maintain, are more amenable to high-throughput studies and are not subjected to the same ethical constraints as vertebrates. In addition to these benefits, *G. mellonella* larvae possess a number of other characteristics which make these organisms particularly useful for the study of human pathogens. Larvae are relatively large in size (12–20 mm) which enables easy manipulation and facilitates the collection of tissue/hemolymph samples for downstream analysis. The immune system of *G. mellonella* larvae share a high degree of structural and functional homology to the innate immune systems of vertebrates and possess both cellular and humoral defenses.1,2 The humoral immune response of insects consists of several processes including melanization, hemolymph clotting, and the production of numerous potent antimicrobial peptides. The cellular response includes phagocytosis, nodulation, and large-scale encapsulation.2,3 Furthermore, *G. mellonella* larvae can be maintained at 37 °C, an important attribute when studying human pathogens that may undergo significant transcriptomic changes at temperatures above or below human body temperature.4

The development of model organisms as research tools in life sciences has been crucial for the advancement of knowledge across many disciplines. Critical to the success of any model organism as a research tool is the standardization of strains and propagation/maintenance conditions to produce organisms with the least possible variation among sources and across generations. Furthermore, it is now widely accepted that model organisms should be amenable to forward-genetic approaches (phenotype to gene) and reverse-genetic approaches (gene to phenotype) facilitated by standard genetic manipulation techniques.5 To accommodate this, current research utilizing model organisms is dependent on organism-specific infrastructure including both stock/strain centers and cyber-infrastructure such as public databases for dissemination of genetic information and results. The highly successful invertebrate models *Caenorhabditis elegans* and *Drosophila melanogaster* have had stock centers and community databases maintained by joint international funding approaches, such as Flybase and WormBase, which have collated data associated with genome sequencing, transcriptomic, and proteomic projects for these organisms.5 This approach has been critical for the successful development of these model organisms.

When compared with *C. elegans* and *D. melanogaster*, the development of *G. mellonella* as a model organism is in its infancy and research in this area does not benefit from access to annotated genomes, established microarrays, RNA interference libraries, or mutant strains which are readily available for other model organisms. Despite this, the pathogenesis of several bacterial and fungal human pathogens has been investigated in *G. mellonella* producing results that correlate closely with those obtained from similar investigations using mammalian host models (Table 1). However, the recent study by Loh et al.,6 in this issue of *Virulence*, which examined the virulence of multiple *Streptococcus pyogenes* serotypes, found strain MGAS315 (a strain that has been well characterized by numerous research groups) to be significantly less pathogenic in *G. mellonella* larvae than what had been published previously in the literature.7 While more studies investigating the virulence of the same strains in different laboratories are required, this observation suggests variation in larvae source, larvae maintenance or experimental conditions could influence the data generated when using *G. mellonella* as a model organism.

As outlined in Table 1, *G. mellonella* larvae are sourced from a wide range of suppliers for virulence studies. Without a standardized source of *G. mellonella* larvae and limited genetic data, it is currently impossible to rule out the influence of genetic variability or epigenetic difference between populations on experimental outcomes. Previous research has shown that genetic variation within populations of *D. melanogaster* influences susceptibility of these organisms to a variety of microbial pathogens.8 With increasing use of *G. mellonella* as a model host for microbial infection, it is becoming more important to examine the immune response of this organism in greater detail by characterizing the genetic aspects of immunity. To compensate for the lack of genomic sequence information in *G. mellonella*, Vogel and colleagues recently subjected the transcriptome of different developmental
Table 1. Differences in environment and experimental conditions during infection of *G. mellonella* larvae with bacterial and fungal pathogens

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Source</th>
<th>Maintenance conditions</th>
<th>Post-treatment conditions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus cereus</em></td>
<td>Not given</td>
<td>Stored at 25 °C, fed beeswax and pollen</td>
<td>Y Oral inoculation, 24 h</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Starved 24 h before infection</td>
<td>N Oral inoculation, 48 h</td>
<td>12</td>
</tr>
<tr>
<td><em>Cryptococcus neoformans</em></td>
<td>Van der Horst Wholesale</td>
<td>Stored in the dark and used within 7 d from the day of shipment</td>
<td>37 °C and 30 °C N 300 h</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>37 °C and at RT 37 °C 14 d</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>37 °C 100 h</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus fumigatus</em></td>
<td>Meal Worm Company</td>
<td>Stored with wood shavings in the dark at 15 °C. Used within 3 wks</td>
<td>30 °C 4–5 d</td>
<td>16 and 17</td>
</tr>
<tr>
<td></td>
<td>Van der Horst Wholesale</td>
<td>Stored with wood shavings in the dark at 22 °C prior to use</td>
<td>37 °C in a moist chamber</td>
<td>18</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em></td>
<td>Sunfish Bait Company</td>
<td>Artificial dietb</td>
<td>22 °C Y Until moribund</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Peterborough Live Bait</td>
<td></td>
<td>30 °C N Topical application, 10 d</td>
<td>20</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>Meal Worm Company</td>
<td>Stored in the dark and used within 7 d from the day of shipment</td>
<td>37 °C 72 h</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Sunfish Bait Company</td>
<td></td>
<td>48 h</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100 h</td>
<td>23</td>
</tr>
<tr>
<td><em>Acinetobacter baumannii</em></td>
<td>Sunfish Bait Company</td>
<td>Stored in the dark and used within 7 d from the day of shipment</td>
<td>37 °C 6 d</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Livefood UK Ltd.</td>
<td>Stored in the dark on woodchips at room temperature</td>
<td>37 °C in dark 24 h</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stored at 15 °C in wood shavings prior to use</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>Larvae reared from eggs</td>
<td>Artificial dietb</td>
<td>30 °C 7 d</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Livefood UK Ltd.</td>
<td>Stored in the dark on woodchips at room temperature</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Injection into hind right proleg, 5 d</td>
<td>30</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Van der Horst Wholesale</td>
<td>N/A</td>
<td>25 °C 60 h</td>
<td>31–33</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>37 °C 4 d</td>
<td>34</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Van der Horst Wholesale</td>
<td>Stored in the dark and used within 7 d from the day of shipment</td>
<td>30 °C and 37 °C 150 h</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Livefood UK Ltd.</td>
<td></td>
<td>25 °C, 30 °C, and 37 °C 120 h</td>
<td>36</td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em></td>
<td>Biosuppliers</td>
<td>Stored at RT in the dark with food, used within 2 weeks</td>
<td>37 °C 5 d</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Best Bet Inc.</td>
<td>Stored in the dark at 10–12 °C and used within 10 d</td>
<td>37 °C and 0.5% CO₂ Y 96 h</td>
<td>7</td>
</tr>
<tr>
<td><em>Enterococcus faecium</em></td>
<td>No source given</td>
<td>Artificial dietb</td>
<td>37 °C 72 h</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Larvae reared from eggs</td>
<td></td>
<td>N/A N 50 h</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>Larvae reared from eggs</td>
<td></td>
<td>37 °C 2–5 d</td>
<td>39</td>
</tr>
</tbody>
</table>

*a* All infections of *G. mellonella* were conducted by injection in the hindmost left proleg at fifth or sixth instar, unless otherwise noted in duration section. *b* Artificial diet = 22% maize meal, 22% wheat germ, 11% dry yeast, 17.5% beeswax, 11% honey, and 11% glycerin.
stages and immune-challenged larvae to next generation sequencing. The data obtained was rich in gene transcripts related to immunity and, in the absence of a genome sequence, will provide a platform for more detailed studies examining molecular mechanisms underlying host-pathogen interactions. Currently, there is little information known about propagation conditions used for G. mellonella larvae and how they differ between global suppliers. As these organisms are not raised under standardized conditions, the different environments used for propagation may influence the natural bacterial flora associated with these larvae which may also influence their susceptibility to infection. Similarly, once G. mellonella larvae are acquired by researchers, the environmental conditions and diet used for maintenance also varies between groups. A recent study by Banville et al.10 showed that larvae deprived of food during experiments while others do not (Table 1). For G. mellonella larvae to be widely accepted as a model organism for the study of microbial pathogenesis, a number of standardization procedures need to be implemented to ensure experimental comparability between different research laboratories. Currently, there are no reference populations of G. mellonella larvae that are available to researchers. Reference populations of strains should be well characterized in terms of sequence, gene function, and phenotype. Additionally, strains should be propagated and maintained by suppliers using standardized and controlled environmental conditions that minimize genetic drift. Where possible, experimental conditions should also be standardized (or at a minimum described in full detail) to allow experiments to be reproduced with minimal ambiguity. Research focused on the collation of data, the standardization of techniques and the dissemination of this information will further advance the usefulness of G. mellonella as a model organism. Without these measures, research utilizing G. mellonella larvae will be restricted to stand alone experiments with only limited scope for inter-laboratory comparisons which will impact upon the development of G. mellonella as a model host for microbial pathogens.

References

17. Scally LR, Bodecky MJ. A cysteine/methionine auxotroph of the opportunistic fungus Aspergillus flavus is associated with host-range restriction: a model for emerging diseases. Microbiology 2006; 152:223-32; PMID:16385132; http://dx.doi.org/10.1099/mic.0.28452-0


30. Joyce SA, Gahan CGM. Molecular pathogenesis of Listeria monocytogenes in the alternative model host Galleria mellonella. Microbiology 2010; 156:3456-68; PMID:20688820; http://dx.doi.org/10.1099/mic.0.040782-0


