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Developing *Galleria mellonella* as a model host for human pathogens

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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, nodulization, 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

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Table 1. Differences in environment and experimental conditions during infection of *G. mellonella* larvae with bacterial and fungal pathogens

Pathogen	Source	Maintenance conditions	Post-treatment conditions			References
			Temperature	Food (Y/N)	Duration[a]	
Bacillus cereus	Not given	Stored at 25 °C, fed beeswax and pollen		Y	Oral inoculation, 24 h	11
		Starved 24 h before infection	37 °C	N	Oral inoculation, 48 h	12
Cryptococcus neoformans	Van der Horst Wholesale	Stored in the dark and used within 7 d from the day of shipment	37 °C and 30 °C	N	300 h	13
			37 °C and at RT		14 d	14
			37 °C		100 h	15
Aspergillus fumigatus	Meal Worm Company	Stored with wood shavings in the dark at 15 °C. Used within 3 wks	30 °C	N	4–5 d	16 and 17
	Van der Horst Wholesale	Stored in wood shavings in the dark at 22 °C prior to use	37 °C in a moist chamber		12 d	18
Aspergillus flavus	Sunfish Bait Company	Artificial diet[b]	22 °C	Y	Until moribund	19
	Peterborough Live Bait		30 °C	N	Topical application, 10 d	20
Candida albicans	Meal Worm Company	Stored in wood shavings in the dark at 15 °C. Used within 3 wks	30 °C	N	72 h	21
					48 h	22
	Sunfish Bait Company	Stored in the dark and used within 7 d from the day of shipment	37 °C		100 h	23
Acinetobacter baumannii	Sunfish Bait Company	Stored in the dark and used within 7 d from the day of shipment	37 °C and 30 °C	N	6 d	24
	Livefood UK Ltd.	Stored in the dark on woodchips at room temperature	37 °C in dark		24 h	25
		Stored at 15 °C in wood shavings prior to use	37 °C		96 h	26
	No source given		37 °C		3 d	27
	Van der Horst Wholesale	Stored in the dark and used within 7 d from the day of shipment	37 °C in dark		6 d	28
Listeria monocytogenes	Larvae reared from eggs	Artificial diet[b]	30 °C	N	7 d	29
	Livefood UK Ltd.	Stored in the dark on woodchips at room temperature	37 °C		Injection into hind right proleg, 5 d	30
Pseudomonas aeruginosa	Van der Horst Wholesale	N/A	25 °C	N	60 h	31–33
			37 °C		4 d	34
Staphylococcus aureus	Van der Horst Wholesale	Stored in the dark and used within 7 d from the day of shipment	30 °C and 37 °C	N	150 h	35
	Livefood UK Ltd.		25 °C, 30 °C, and 37 °C		120 h	36
Streptococcus pyogenes	Biosuppliers	Stored at RT in the dark with food, used within 2 weeks	37 °C	N	5 d	6
	Best Bet Inc.	Stored in the dark at 10–12 °C and used within 10 d	37 °C and 0.5% CO_2	Y	96 h	7
Enterococcus faecium	No source given	Artificial diet[b]	37 °C	N	72 h	37
	Larvae reared from eggs		N/A		50 h	38
	Larvae reared from eggs	Stored at 25 °C, fed beeswax and pollen	37 °C		2–5 d	39

[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.[9] 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 nutrition for seven days prior to infection were more susceptible to infection with the fungal pathogen *C. albicans*.[10] Starved larvae demonstrated reduced expression of a variety of antimicrobial peptides and immune proteins.[10] This finding has implications for inter-laboratory comparisons of virulence studies using *G. mellonella* as some experimental protocols indicate that larvae are maintained with 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

1. Lionakis MS. Drosophila and Galleria insect model hosts: new tools for the study of fungal virulence, pharmacology and immunology. Virulence 2011; 2:521-7; PMID:22186764; http://dx.doi.org/10.4161/viru.2.6.18520
2. Hoffmann JA. Innate immunity of insects. Curr Opin Immunol 1995; 7:4-10; PMID:7772280; http://dx.doi.org/10.1016/0952-7915(95)80022-0
3. Kavanagh K, Reeves EP. Exploiting the potential of insects for in vivo pathogenicity testing of microbial pathogens. FEMS Microbiol Rev 2004; 28:101-12; PMID:14975532; http://dx.doi.org/10.1016/j.femsre.2003.09.002
4. Smoot LM, Smoot JC, Graham MR, Somerville GA, Sturdevant DE, Migliaccio CAL, et al. Global differential gene expression in response to growth temperature alteration in group A Streptococcus. Proc Natl Acad Sci U S A 2001; 98:10416-21; PMID:11517341; http://dx.doi.org/10.1073/pnas.191267598
5. Ankeny RA, Leonelli S. What's so special about model organisms? Stud Hist Philos Sci 2011; 42:313-23; http://dx.doi.org/10.1016/j.shpsa.2010.11.039
6. Loh JMS, Adenwalla N, Wiles S, Proft T. *Galleria mellonella* larvae as an infection model for group A streptococcus. Virulence 2013; 4:419-28; PMID:23652836; http://dx.doi.org/10.4161/viru.24930.
7. Olsen RJ, Watkins ME, Cantu CC, Beres SB, Musser JM. Virulence of serotype M3 Group A Streptococcus strains in wax worms (Galleria mellonella larvae). Virulence 2011; 2:111-9; PMID:21258213; http://dx.doi.org/10.4161/viru.2.2.14338
8. Tinsley MC, Blanford S, Jiggins FM. Genetic variation in Drosophila melanogaster pathogen susceptibility. Parasitology 2006; 132:767-73; PMID:16497252; http://dx.doi.org/10.1017/S0031182006009929
9. Vogel H, Altincicek B, Glöckner G, Vilcinskas A. A comprehensive transcriptome and immune-gene repertoire of the lepidopteran model host Galleria mellonella. BMC Genomics 2011; 12:308; PMID:21663692; http://dx.doi.org/10.1186/1471-2164-12-308
10. Banville N, Browne N, Kavanagh K. Effect of nutrient deprivation on the susceptibility of Galleria mellonella larvae to infection. Virulence 2012; 3:497-503; PMID:23076277; http://dx.doi.org/10.4161/viru.21972
11. Fedhila S, Daou N, Lereclus D, Nielsen-LeRoux C. Identification of Bacillus cereus internalin and other candidate virulence genes specifically induced during oral infection in insects. Mol Microbiol 2006; 62:339-55; PMID:16978259; http://dx.doi.org/10.1111/j.1365-2958.2006.05362.x
12. Kamar R, Gohar M, Jéhanno I, Réjasse A, Kallassy M, Lereclus D, et al. Pathogenic potential of Bacillus cereus strains as revealed by phenotypic analysis. J Clin Microbiol 2013; 51:320-3; PMID:23135929; http://dx.doi.org/10.1128/JCM.02848-12
13. Mylonakis E, Moreno R, El Khoury JB, Idnurm A, Heitman J, Calderwood SB, et al. Galleria mellonella as a model system to study Cryptococcus neoformans pathogenesis. Infect Immun 2005; 73:3842-50; PMID:15972469; http://dx.doi.org/10.1128/IAI.73.7.3842-3850.2005
14. Velagapudi R, Hsueh YP, Geunes-Boyer S, Wright JR, Heitman J. Spores as infectious propagules of Cryptococcus neoformans. Infect Immun 2009; 77:4345-55; PMID:19620339; http://dx.doi.org/10.1128/IAI.00542-09
15. Fuchs BB, O'Brien E, Khoury JB, Mylonakis E. Methods for using Galleria mellonella as a model host to study fungal pathogenesis. Virulence 2010; 1:475-82; PMID:21178491; http://dx.doi.org/10.4161/viru.1.6.12985
16. Reeves EP, Messina CGM, Doyle S, Kavanagh K. Correlation between gliotoxin production and virulence of Aspergillus fumigatus in Galleria mellonella. Mycopathologia 2004; 158:73-9; PMID:15487324; http://dx.doi.org/10.1023/B:MYCO.0000038434.55764.16
17. Renwick J, Daly P, Reeves EP, Kavanagh K. Susceptibility of larvae of Galleria mellonella to infection by Aspergillus fumigatus is dependent upon stage of conidial germination. Mycopathologia 2006; 161:377-84; PMID:16761185; http://dx.doi.org/10.1007/s11046-006-0021-1
18. Jackson JC, Higgins LA, Lin XR. Conidiation color mutants of Aspergillus fumigatus are highly pathogenic to the heterologous insect host Galleria mellonella. PLoS One 2009; 4:e4224; PMID:19156203; http://dx.doi.org/10.1371/journal.pone.0004224
19. St Leger RJ, Screen SE, Shams-Pirzadeh B. Lack of host specialization in Aspergillus flavus. Appl Environ Microbiol 2000; 66:320-4; PMID:10618242; http://dx.doi.org/10.1128/AEM.66.1.320-324.2000
20. Scully LR, Bidochka 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
21. Cotter G, Doyle S, Kavanagh K. Development of an insect model for the in vivo pathogenicity testing of yeasts. FEMS Immunol Med Microbiol 2000; 27:163-9; PMID:10640612; http://dx.doi.org/10.1111/j.1574-695X.2000.tb01427.x
22. Brennan M, Thomas DY, Whiteway M, Kavanagh K. Correlation between virulence of Candida albicans mutants in mice and Galleria mellonella larvae. FEMS Immunol Med Microbiol 2002; 34:153-7; PMID:12381467; http://dx.doi.org/10.1111/j.1574-695X.2002.tb00617.x
23. Fuchs BB, Eby J, Nobile CJ, El Khoury JB, Mitchell AP, Mylonakis E. Role of filamentation in Galleria mellonella killing by Candida albicans. Microbes Infect 2010; 12:488-96; PMID:20223293; http://dx.doi.org/10.1016/j.micinf.2010.03.001
24. Peleg AY, Jara S, Monga D, Eliopoulos GM, Moellering RC Jr., Mylonakis E. Galleria mellonella as a model system to study Acinetobacter baumannii pathogenesis and therapeutics. Antimicrob Agents Chemother 2009; 53:2605-9; PMID:19332683; http://dx.doi.org/10.1128/AAC.01533-08
25. Wand ME, Bock LJ, Turton JF, Nugent PG, Sutton JM. Acinetobacter baumannii virulence is enhanced in Galleria mellonella following biofilm adaptation. J Med Microbiol 2012; 61:470-7; PMID:22194338; http://dx.doi.org/10.1099/jmm.0.037523-0
26. Hornsey M, Wareham DW. In vivo efficacy of glycopeptide-colistin combination therapies in a Galleria mellonella model of Acinetobacter baumannii infection. Antimicrob Agents Chemother 2011; 55:3534-7; PMID:21502628; http://dx.doi.org/10.1128/AAC.00230-11

27. Antunes LCS, Imperi F, Carattoli A, Visca P. Deciphering the multifactorial nature of Acinetobacter baumannii pathogenicity. PLoS One 2011; 6:e22674; PMID:21829642; http://dx.doi.org/10.1371/journal.pone.0022674
28. Gaddy JA, Arivett BA, McConnell MJ, López-Rojas R, Pachón J, Actis LA. Role of acinetobactin-mediated iron acquisition functions in the interaction of Acinetobacter baumannii strain ATCC 19606T with human lung epithelial cells, Galleria mellonella caterpillars, and mice. Infect Immun 2012; 80:1015-24; PMID:22232188; http://dx.doi.org/10.1128/IAI.06279-11
29. Mukherjee K, Altincicek B, Hain T, Domann E, Vilcinskas A, Chakraborty T. Galleria mellonella as a model system for studying Listeria pathogenesis. Appl Environ Microbiol 2010; 76:310-7; PMID:19897755; http://dx.doi.org/10.1128/AEM.01301-09
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
31. Jander G, Rahme LG, Ausubel FM. Positive correlation between virulence of Pseudomonas aeruginosa mutants in mice and insects. J Bacteriol 2000; 182:3843-5; PMID:10851003; http://dx.doi.org/10.1128/JB.182.13.3843-3845.2000
32. Choi JY, Sifri CD, Goumnerov BC, Rahme LG, Ausubel FM, Calderwood SB. Identification of virulence genes in a pathogenic strain of Pseudomonas aeruginosa by representational difference analysis. J Bacteriol 2002; 184:952-61; PMID:11807055; http://dx.doi.org/10.1128/jb.184.4.952-961.2002
33. Hendrickson EL, Plotnikova J, Mahajan-Miklos S, Rahme LG, Ausubel FM. Differential roles of the Pseudomonas aeruginosa PA14 rpoN gene in pathogenicity in plants, nematodes, insects, and mice. J Bacteriol 2001; 183:7126-34; PMID:11717271; http://dx.doi.org/10.1128/JB.183.24.7126-7134.2001
34. Miyata S, Casey M, Frank DW, Ausubel FM, Drenkard E. Use of the Galleria mellonella caterpillar as a model host to study the role of the type III secretion system in Pseudomonas aeruginosa pathogenesis. Infect Immun 2003; 71:2404-13; PMID:12704110; http://dx.doi.org/10.1128/IAI.71.5.2404-2413.2003
35. Peleg AY, Monga D, Pillai S, Mylonakis E, Moellering RC Jr., Eliopoulos GM. Reduced susceptibility to vancomycin influences pathogenicity in Staphylococcus aureus infection. J Infect Dis 2009; 199:532-6; PMID:19125671; http://dx.doi.org/10.1086/596511
36. Desbois AP, Coote PJ. Wax moth larva (Galleria mellonella): an in vivo model for assessing the efficacy of antistaphylococcal agents. J Antimicrob Chemother 2011; 66:1785-90; PMID:21622972; http://dx.doi.org/10.1093/jac/dkr198
37. Lebreton F, van Schaik W, Sanguinetti M, Posteraro B, Torelli R, Le Bras F, et al. AsrR is an oxidative stress sensing regulator modulating Enterococcus faecium opportunistic traits, antimicrobial resistance, and pathogenicity. PLoS Pathog 2012; 8:e1002834; PMID:22876178; http://dx.doi.org/10.1371/journal.ppat.1002834
38. Park IJ, Lee WG, Lim YA, Cho SR. Genetic rearrangements of TN1546-like elements in vancomycin-resistant Enterococcus faecium isolates collected from hospitalized patients over a seven-year period. J Clin Microbiol 2007; 45:3903-8; PMID:17898158; http://dx.doi.org/10.1128/JCM.01085-07
39. Gaspar F, Teixeira N, Rigottier-Gois L, Marujo P, Nielsen-LeRoux C, Crespo MTB, et al. Virulence of Enterococcus faecalis dairy strains in an insect model: the role of fsrB and gelE. Microbiology 2009; 155:3564-71; PMID:19696101; http://dx.doi.org/10.1099/mic.0.030775-0