Porphyromonas gingivalis Virulence in a Drosophila melanogaster Model

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Porphyromonas gingivalis has been implicated in the etiology of adult periodontitis. In this study, we examined the viability of Drosophila melanogaster as a new model for examining P. gingivalis-host interactions. P. gingivalis (W83) infection of Drosophila resulted in a systemic infection that killed in a dose-dependent manner. Differences in the virulence of several clinically prevalent P. gingivalis strains were observed in the Drosophila killing model, and the results correlated well with studies in mammalian infection models and human epidemiological studies. P. gingivalis pathobiology in Drosophila did not result from uncontrolled growth of the bacterium in the Drosophila hemolymph (blood) or overt damage to Drosophila tissues. P. gingivalis killing of Drosophila was multifactorial, involving several bacterial factors that are also involved in virulence in mammals. The results from this study suggest that many aspects of P. gingivalis pathogenesis in mammals are conserved in Drosophila, and thus the Drosophila killing model should be useful for characterizing P. gingivalis-host interactions and, potentially, polymicrobe-host interactions.

Porphyromonas gingivalis is a Gram-negative, obligate anaerobe that has been strongly implicated in the etiology of adult (chronic) periodontitis (21, 29), a destructive disease that affects the gums and supporting structures of the teeth. A. (chronic) periodontitis (21, 29), a destructive disease that affects the gums and supporting structures of the teeth. Periodontal bone loss (20, 40) and soft tissue destruction (6, 11, 31, 35).

The fruit fly, Drosophila melanogaster, has been well established as a nonmammalian model for studying host-pathogen interactions (1, 17, 52, 55, 63). Drosophila relies solely on an innate immune response to combat invading microbes, and this immune response strongly parallels the mammalian innate immune response (47, 48). Like the mammalian innate immune response, Drosophila uses pattern recognition receptors to detect conserved microbial motifs on invading microbes, and the flies activate an immune response that is specific for the type of invading microbe. The absence of an adaptive immune response makes Drosophila useful for studying the interactions between microbes and the host innate immune response, in isolation. Numerous tools exist for the genetic manipulation of Drosophila, and these have been used to generate thousands of transgenic and mutant lines, including lines useful for identifying host factors that promote or fight infection (9, 52). The Drosophila genome has been sequenced, which has facilitated the development of microarray, proteomic, and RNA interference (RNAi) technologies for genome-wide analysis of Drosophila processes (14, 23, 65, 66). Additionally, the relative affordability, short generation time (10 to 14 days), and ease of use allow sample sizes that are large enough to permit statistical analysis of the data and make Drosophila an attractive complement to mammalian models.

The Drosophila killing model has been successfully used to characterize host-microbe interactions of bacterial (9, 13, 15, 17, 22, 52, 55), fungal (1, 3), and parasitic (62) pathogens, and the results of these studies suggest that there is good correlation between pathogenesis in mammals and in nonmammalian animals such as Drosophila. In these studies the microbe of interest is either fed to Drosophila or introduced directly into the hemocoel (body cavity) through the thorax by using a needle, and the survival of the infected animals is monitored over time.

P. gingivalis grows optimally at 37°C, and in contrast to other bacteria studied using the Drosophila model, it (and other oral pathogens) is an obligate anaerobe. It was therefore unclear whether this model would be viable for studying P. gingivalis-host interactions. The objective of the current study was to determine the viability of the Drosophila killing model for studying P. gingivalis-host interactions. We demonstrate that P. gingivalis is pathogenic in Drosophila, killing the animals in a dose-dependent manner. P. gingivalis killing of Drosophila was not due to uncontrolled bacterial growth or overt damage to Drosophila tissues. Heat-killed and live P. gingivalis microorganisms were equally pathogenic in Drosophila, which suggests that P. gingivalis cell surface components and Drosophila immune responses play important roles in causing pathology in this model. Differences in the virulence of several clinically prevalent P. gingivalis strains were observed using the Drosophila killing model and correlated well with studies in mammalian infection models (24, 28, 43, 56) and human epidemiological studies (30). Additionally, multiple P. gingivalis components...
that are involved in virulence in mammals were found to be involved in Drosophila killing.

MATERIALS AND METHODS

**Bacterial and Drosophila strains and growth conditions.** Bacterial and Drosophila strains used in this study are described in Table 1. P. gingivalis strains were grown on brucella blood agar (BBA; Anaerobe Systems), at 37°C in an anaerobic chamber (85% N₂, 10% H₂, 5% CO₂). Drosophila stocks were maintained and propagated at 26°C in standard culture vials containing corn flour-molasses medium. Only 3- to 5-day-old female flies were used in experiments.

**Infection of adult female Drosophila.** Bacterial strains were grown in 40 ml of Trypticase soy broth (TSB) for 24 h at 37°C. *Pseudomonas aeruginosa* and *Escherichia coli* were grown aerobically with shaking. *P. gingivalis* was supplemented with hemin (5 μg/ml) and vitamin K (1 μg/ml) and incubated anaerobically. The bacteria were harvested by centrifugation at 4,000 rpm for 8 min and diluted in TSB to the following optical densities at 600 nm (OD₆₀₀; unless otherwise noted): *P. aeruginosa*, 0.7; *E. coli DH5α*, 2.0 (1 × 10⁹ CFU ml⁻¹); P. gingivalis, 2.0 (1.09 × 10⁺ CFU ml⁻¹). For heat killing, *P. gingivalis* was incubated at 60°C for 1 min. To confirm loss of viability, the cultures were grown on BBA to determine the number of colony forming units (CFU) on plates containing gentamicin (8 μg/ml), amphotericin B (3 μg/ml), and vancomycin (1 μg/ml). The experiment was repeated, and the results were averaged.

**Statistical methods.** (i) Sample size. Power calculations based on pilot data estimated that a sample size of 136 Drosophila flies per group would be sufficient to detect a relative risk of mortality (RR) of at least 2.0 at an α-level of 0.05 with 90% power when comparing different infections. A sample size of 150 animals per group was used unless otherwise noted. Depending on the number of experimental groups involved, each experiment was divided into three, four, or five parts for feasibility. (ii) Data analysis. Survival data were analyzed using the SAS statistical software package (SAS Institute, Cary, NC). A Cox proportional hazards (P-H) model was fitted to the survival data. Likelihood ratio tests were performed, and RR values were obtained from the fitted Cox P-H model and adjusted for the individual “experiments” and “parts.” RR values with P values of <0.05 were considered significant.

**RESULTS**

*P. gingivalis* pathogenesis in the Drosophila killing model. To determine whether *P. gingivalis* is pathogenic in *Drosophila*, groups of adult female *Drosophila* flies were infected with *P. gingivalis* strain W83, a *P. aeruginosa* strain (PA01) that was previously shown to be highly pathogenic (19), an *E. coli* strain (DH5α) that was previously shown to be nonpathogenic (15), or mock infected. The survival curves of infected and mock-infected *Drosophila* are shown in Fig. 1. *Drosophila* animals infected with *P. gingivalis* strain W83 were significantly more likely to die than mock-infected animals (RR for W83 versus mock, 3.58; P = 0.0038) and *E. coli* DH5α-infected animals (RR for W83 versus DH5α, 2.78; P = 0.01) but significantly less likely to die than *P. aeruginosa*-infected animals (RR for W83 versus PA01, 0.39; P = 0.0012). To determine whether *P. gingivalis* kills *Drosophila* in a dose-dependent manner, groups of *Drosophila* flies were infected with increasing concentrations of *P. gingivalis* strain W83. The survival curves of *Drosophila* flies infected with different amounts of strain W83 are shown in Fig. 2. An increase in the amount of *P. gingivalis* that was inoculated into *Drosophila* flies per group were infected (as described above) with the following *P. gingivalis* strains: W83, ATCC 49417, 381, ATCC 33277, A7A1, and 23A4. Three animals per group were ground in 80 μl of PBS with a Teflon pestle at the following time points (in hours) postinfection: 0, 4, 8, 12, 24, 36, 48, 60, 72, 84, 96, 108, and 120. Viable *P. gingivalis* cell counts were determined on BBA plates containing gentamicin (8 μg/ml), amphotericin B (3 μg/ml), and vancomycin (1 μg/ml). The experiment was repeated, and the results were averaged.

**TABLE 1. Bacterial and Drosophila strains used in this study**

<table>
<thead>
<tr>
<th>Strain or stock</th>
<th>Description or genotype</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. gingivalis</em> strains</td>
<td>Lab strain, originally isolated from an oral infection</td>
<td>Margaret Duncan</td>
</tr>
<tr>
<td>W83</td>
<td>Lab strain, originally isolated from an oral infection</td>
<td>Joseph Zambon</td>
</tr>
<tr>
<td>ATCC 49417</td>
<td>Lab strain, originally isolated from an oral infection</td>
<td>Joseph Zambon</td>
</tr>
<tr>
<td>381</td>
<td>Lab strain, originally isolated from an oral infection</td>
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</tr>
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<td>ATCC 33277</td>
<td>Lab strain, originally isolated from an oral infection</td>
<td>Joseph Zambon</td>
</tr>
<tr>
<td>A7A1</td>
<td>Lab strain, originally isolated from an oral infection</td>
<td>Mike Curtis</td>
</tr>
<tr>
<td>23A4</td>
<td>Lab strain, renamed W50UK for these studies; wt for E8 and GPC</td>
<td>Mike Curtis</td>
</tr>
<tr>
<td>W50</td>
<td>Lab strain; renamed W83VA for these studies; wt for V2577</td>
<td>Janina Lewis</td>
</tr>
<tr>
<td>E8</td>
<td>rpmA rpmB double mutant (arginine-specific protease)</td>
<td>Mike Curtis</td>
</tr>
<tr>
<td>GPC</td>
<td>Capsule mutant</td>
<td>Mike Curtis</td>
</tr>
<tr>
<td>W83 2741</td>
<td>Lab strain, renamed W83VA for these studies; wt for V2577</td>
<td>Janina Lewis</td>
</tr>
<tr>
<td>V2577</td>
<td>kgp mutant (lysine-specific protease)</td>
<td>Janina Lewis</td>
</tr>
<tr>
<td>ATCC 33277</td>
<td>Lab strain, renamed 33277FL for these studies; wt for YPF1 and SMF1</td>
<td>Richard Lamont</td>
</tr>
<tr>
<td>YPF1</td>
<td>fimA mutant (major fimbrae)</td>
<td>Richard Lamont</td>
</tr>
<tr>
<td>SMF1</td>
<td>mfa1 mutant (minor fimbrae)</td>
<td>Richard Lamont</td>
</tr>
<tr>
<td>W83</td>
<td>Lab strain, renamed W833P for these studies; wt for M1217</td>
<td>Koji Nakayama</td>
</tr>
<tr>
<td>M1217</td>
<td>mgl mutant (L-methionine-α-deamino-γ-mercaptomethane-lyase)</td>
<td>Koji Nakayama</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> PA01</td>
<td>Lab strain</td>
<td>Neil Baker</td>
</tr>
<tr>
<td>E. coli DH5α</td>
<td></td>
<td>Invitrogen</td>
</tr>
<tr>
<td><em>D. melanogaster</em> Canton S</td>
<td>Wild type</td>
<td>Amanda Simcox</td>
</tr>
</tbody>
</table>
resulted in an increase in the number of animals that were killed during the time course of the experiment.

To determine whether \textit{P. gingivalis} spreads systemically once introduced into \textit{Drosophila}, animals were infected with FITC-labeled strain W83 or mock infected and examined using fluorescence microscopy 2 h postinfection. As there were no fluorescent \textit{P. gingivalis} reference strains, FITC labeling was used. Representative bright-field and FITC images of \textit{P. gingivalis}- and mock-infected animals are shown in Fig. 3. By 2 h postinfection strong FITC fluorescence was observed at the site of inoculation (white arrows), and the fluorescent bacteria were detected throughout the abdomen of the animals (Fig. 3B and D, gray arrows), as has been observed with other bacterial infections in \textit{Drosophila} (22, 55). This was not surprising given the open circulatory system of \textit{Drosophila} and the direct inoculation of the bacteria into the hemocoel. FITC fluorescence was not observed in mock-infected animals (Fig. 3F and H).

**Virulence of \textit{P. gingivalis} strains in the \textit{Drosophila} killing model.** To compare the virulence of several clinically prevalent \textit{P. gingivalis} strains, \textit{Drosophila} animals were infected with strains W83, ATCC 49417, ATCC 33277, 381, A7A1, or 23A4 or mock infected. The \textit{P. gingivalis} strains that were used in this experiment are commonly used lab strains that were originally isolated from individuals with oral infections, and they were selected for this study because they were identified in previous epidemiologic studies as being prevalent in human populations (30, 49). The survival curves for animals infected with each of the \textit{P. gingivalis} test strains are shown in Fig. 4. The RR values for pairwise comparisons of the \textit{P. gingivalis} strain infections are shown in Table 2. Based on the RR values, \textit{Drosophila} animals were significantly more likely to die from infection with any of the \textit{P. gingivalis} test strains than from a mock infection. \textit{Drosophila} animals were significantly more likely to die from a W83 infection than from an infection with any of the

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**FIG. 1.** Survival curves of adult, female \textit{Drosophila} flies infected with \textit{P. gingivalis} and other bacterial species. Eighteen \textit{Drosophila} flies per group were infected with \textit{P. gingivalis} (W83), \textit{P. aeruginosa} (PAO1), or \textit{E. coli} DH5α or mock infected, and the number of dead animals was recorded every 10 h for a period of 130 h. The experiment was repeated for a total sample size of 36 animals per group.

**FIG. 2.** Survival curves of adult female \textit{Drosophila} flies infected with different amounts of \textit{P. gingivalis} (W83). Thirty \textit{Drosophila} flies per group were infected with strain W83 at an OD_{600} of 0.5, 1.0, or 2.0 or were mock infected. The experiment was repeated for a total sample size of 60 animals per group.
other *P. gingivalis* test strains, and they were also more likely to die from an A7A1 infection than from a 23A4 infection.

To examine the growth of *P. gingivalis* strains within infected *Drosophila*, colony counts were used to determine the level of viable *P. gingivalis* present in infected animals at specific time points postinfection. *P. gingivalis* colony-forming units that were recovered per animal at several time points are shown in Fig. 5. Based on the number of bacteria recovered immediately after infection (time point zero), between $5.10^{10}$ and $3.17 \times 10^4$ CFU of *P. gingivalis* were inoculated into the animals. Although small increases in the amount of *P. gingivalis* recovered per *Drosophila* were sometimes observed, there was an overall downward trend in the amount of bacteria recovered from the animals over time. A similar trend was observed when real-time PCR was used to quantitate *P. gingivalis* DNA within the animals (data not shown). Significant numbers of viable *P. gingivalis* could persist in the animals postinfection, which was not surprising, as the bacterium exhibits a high degree of aerotolerance and shows no loss of viability after exposure to air for at least 5 h (54). Viable *P. gingivalis* could be recovered.

![FIG. 3. Location of FITC-labeled *P. gingivalis* within infected *Drosophila* animals. *Drosophila* animals were infected with FITC-labeled *P. gingivalis* strain W83 or mock infected and observed 2 h postinfection by using bright-field and fluorescence microscopy. (A to D) *P. gingivalis*-infected animals; (E to H) mock-infected flies. White arrows indicate sites of septic injury in the thorax. Gray arrows indicate FITC-labeled *P. gingivalis* within the abdomens of the infected animals.](https://iai.asm.org/article-static/442/000035-05002017.pdf)

![FIG. 4. Survival curves of adult female *Drosophila* flies infected with several *P. gingivalis* strains. *Drosophila* animals were infected with *P. gingivalis* strain W83, ATCC 49417, ATCC 33277, 381, A7A1, or 23A4 or were mock infected.](https://iai.asm.org/article-static/442/000035-05002017.pdf)
from the *Drosophila* up to 24 h postinfection for strain 23A4, up to 48 h postinfection for strain ATCC 49417, and up to 60 h postinfection for strains W83, 381, ATCC 33277, and A7A1.

To investigate the mechanism of *P. gingivalis* killing of *Drosophila*, the effect of *P. gingivalis* infection on the viability of *Drosophila* tissues was examined, and the survival of animals infected with live versus heat-killed bacteria was compared. Animals infected with strain W83 were dissected, and the viability of their tissues was assessed using the vital dye trypan blue and a LIVE/DEAD cytotoxicity assay. No gross differences in tissue integrity were observed between *P. gingivalis*-and mock-infected animals (data not shown). W83 was heat killed at 60°C for 1 h, which also inactivates the proteases (gingipains) (64). When the survival rates of *Drosophila* animals infected with live and heat-killed W83 were compared (Fig. 6), no differences were observed in the survival of the two groups of animals.

**Effects of *P. gingivalis* putative virulence factor gene knockouts on *Drosophila* survival.** To determine whether similar mechanisms are involved in *P. gingivalis* virulence in mammals and in *Drosophila* and whether multiple *P. gingivalis* factors are involved in *Drosophila* killing, the virulence levels of the following *P. gingivalis* mutants were compared to their wild-type (wt) parental strains using the *Drosophila* model: *rgpA, rgpB* deficient (arginine-specific proteases), *kgp* deficient (lysine-specific protease), *fimA* deficient (fimbrillin, major fimbriae), *mfa1* deficient (minor fimbriae), capsule deficient, and *mgl* deficient (L-methionine-α-deamino-γ-mercaptopropionate-lyase) (Table 1). Groups of wt *Drosophila* were infected with the *P. gingivalis* mutant strains, the corresponding wt strains, or mock infected, and the survival rates of the infected animals were compared.

Survival curves for wt *Drosophila* infected with the wt or mutant *P. gingivalis* strains are shown in Fig. 7. The RR values for pairwise comparisons of infections with wt versus mutant strains of *P. gingivalis* are shown in Table 3. An RR value greater than 1 indicates that *Drosophila* animals were more likely to die from infection with the wt *P. gingivalis* strain than from an infection with the mutant strain. *Drosophila* animals infected with *P. gingivalis* *rgpA, rgpB, kgp, fimA, mfa1*, and capsule mutants were less likely to die than *Drosophila* animals infected with the corresponding wt strains (Fig. 7A to E and

![Graph showing growth of *P. gingivalis* in adult female *Drosophila* flies.](image-url)

**FIG. 5.** Growth of *P. gingivalis* in adult female *Drosophila* flies. *Drosophila* flies were infected with *P. gingivalis* strain W83, ATCC 49417, ATCC 33277, 381, A7A1, or 23A4, and viable cell counts were determined on BBA. Each data point represents the log_{10} value of the average CFU per animal recovered at a specific time point postinfection. Trend lines are shown as dashed lines.
Table 3). *Drosophila* flies infected with the *P. gingivalis* mgl mutant were as likely to die as *Drosophila* flies infected with the wt strain (Fig. 7F and Table 3). The experiments were repeated using a 1:2 dilution of each wt and mutant strain, and similar results were observed (data not shown).

**DISCUSSION**

The goal of this study was to examine the viability of *Drosophila melanogaster* as a model for examining *P. gingivalis*-host interactions. This model has been used to examine other bacteria-host interactions; however, *P. gingivalis* and other oral pathogens are obligate anaerobes and grow optimally at 37°C. The first step was to determine whether *P. gingivalis* was pathogenic in *Drosophila*. When the survival of animals infected with *P. gingivalis* (represented by strain W83) was compared to the survival of animals infected with a known pathogen (*P. aeruginosa* PA01) (19) and of a nonpathogen (*E. coli* DH5α) (15) for *Drosophila*, the *P. gingivalis*-infected animals were significantly more likely to die than *E. coli* DH5α-infected animals, although they were significantly less likely to die than *P. aeruginosa*-infected animals. A dose response was observed for *P. gingivalis* killing of *Drosophila* (Fig. 2), indicating that the bacteria could be more pathogenic at higher infective doses; however, at higher concentrations (ODs greater than 2.0) the bacteria clumped on the needle, making the delivery of higher doses unfeasible.

All *P. gingivalis* test strains were pathogenic to some degree in the *Drosophila* killing model (Table 2). Strain W83 was significantly more pathogenic than the five other *P. gingivalis* strains in this model, which correlates with the observations from studies using rodent models, in which W83 was also more pathogenic than the five other *P. gingivalis* strains (24, 28, 43, 56). W83 is also the strain that is most strongly associated with human periodontitis (30). These results suggest that there is a correlation between *P. gingivalis* pathogenesis in mammals and in *Drosophila*.

*P. gingivalis* is normally exposed to temperatures that range from 37°C to 38.8°C in the oral cavity (2) and grows optimally at 37°C in vitro, and unlike other bacteria studied using the *Drosophila* model, *P. gingivalis* is an obligate anaerobe. Thus, we determined whether *P. gingivalis* could grow in *Drosophila*. Colony counts were used to examine the growth of the *P. gingivalis* strains in the animals, and *P. gingivalis* DNA recovered from infected *Drosophila* was measured by real-time PCR. The results from the growth experiments indicated that although *P. gingivalis* does not multiply effectively in the *Drosophila* body cavity, the bacterium can persist for up to 60 h postinoculation in the animals. The aerobic environment of the *Drosophila* hemolymph and the temperature at which the infected animals are incubated are the likely factors that prevented the bacterium from growing optimally. The infected animals were incubated at 30°C, as their physiological processes deteriorate at temperatures higher than 30°C (42). When W83 growth at 30°C and 37°C was compared, the bacterium grew poorly at 30°C on solid medium (BBA) and in TSB (data not shown).

The six *P. gingivalis* factors tested for a role in *Drosophila* killing have been demonstrated to contribute to the bacterium's virulence in mammals (43, 59, 70, 72). In the current study, five of the components were shown to contribute to *P. gingivalis* virulence in the *Drosophila* model. The gingipains are proteases encoded by *rgpA* (arginine-specific protease), *rgpB* (arginine-specific protease), and *kgp* (lysine-specific protease) genes. They are considered to be major *P. gingivalis* virulence factors due to their ability to degrade a large variety of host proteins (reviewed in references 37, 58, and 60), and they are important to various degrees for pathogenesis in the mouse abscess model of infection (59). *P. gingivalis* major fimbriae (*fimA*) are involved in the invasion of gingival epithelial cells (71), and both major and minor (*mfa1*) fimbriae are important for *P. gingivalis* autoaggregation (50, 57) and biofilm formation with *Streptococcus gordonii* (44, 45). *mfa1* and *fimA* mutants are impaired in the ability to induce periodontal bone loss in a rat model of infection (70). The capsule, which is present on some strains of *P. gingivalis*, is important for virulence in the mouse abscess model of infection, as infection with encapsu-
lated strains results in more soft tissue destruction and death than infection with unencapsulated strains (43). L-Methionine-deamino-mercaptomethane-lyase (mgl) catalyzes the conversion of L-methionine to the volatile sulfur compound methyl mercaptan (72). Methyl mercaptan is elevated in the mouths (68) and crevicular air (12) of patients with active periodontitis, and exposure to methyl mercaptan has been shown to decrease DNA (38) and protein (38, 46) synthesis in gingival fibroblasts and to inhibit the migration of periodontal ligament cells (46). In the mouse abscess model, the mgl mutant was less

![Survival curves of wt Drosophila animals infected with wt or mutant strains of P. gingivalis. Green curves, wt-infected animals; red curves, mutant-infected animals; blue curves, mock-infected animals.](image)

**TABLE 3.** Relative risk of mortality for pairwise comparisons of wt versus mutant *P. gingivalis* infections in *Drosophila*

<table>
<thead>
<tr>
<th>Mutant</th>
<th>wt</th>
<th>RR (P value) for wt vs mutant</th>
<th>Findings in other animal model studies (reference[s]) for:</th>
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<tr>
<td></td>
<td></td>
<td>RR (P value) for wt vs mutant</td>
<td>Periodontal bone loss</td>
</tr>
<tr>
<td>rgsA rgsB mutant (arginine-specific proteases)</td>
<td>W50UK</td>
<td>7.69 (&lt;0.0001)</td>
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</tr>
<tr>
<td>kgs mutant (lysine-specific protease)</td>
<td>W83VA</td>
<td>1.63 (&lt;0.0001)</td>
<td>–</td>
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<tr>
<td>fimA mutant (fimbrillin, major fimbrae)</td>
<td>33277FL</td>
<td>2.13 (&lt;0.0001)</td>
<td>Correlation (14, 46)</td>
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<tr>
<td>mfa1 mutant (minor fimbrae)</td>
<td>33277FL</td>
<td>1.56 (0.0018)</td>
<td>Correlation (46)</td>
</tr>
<tr>
<td>Capsule mutant</td>
<td>W50UK</td>
<td>2.99 (&lt;0.0001)</td>
<td>–</td>
</tr>
<tr>
<td>mgl mutant</td>
<td>W83JP</td>
<td>0.87 (0.2)</td>
<td>–</td>
</tr>
</tbody>
</table>

a RR values with P values of <0.05 are shown in bold.

b –, the strains have not been compared in the indicated model.
able to cause soft tissue destruction and death than the wt strain (72). The results of this study demonstrate that several *P. gingivalis* factors (capsule, major and minor fimbriae, and gingipains) that are important for pathogenesis in mammals are also involved in the killing of *Drosophila*, suggesting that there is a strong correlation between *P. gingivalis* pathogenesis in mammals and in *Drosophila*. However, the noninvolvement of the *mgl* gene product in *P. gingivalis* killing of *Drosophila* suggests that the requirement for a particular virulence factor by *P. gingivalis* depends on the host involved. The observed differences in the *Drosophila*-killing abilities of the *P. gingivalis* strains suggest that additional bacterial factors are involved in pathogenesis in this model.

In contrast to other bacteria that have been studied in this model, *P. gingivalis* does not multiply effectively in *Drosophila*, yet the bacterium still kills the animals. Additionally, no gross differences in tissue integrity were observed between infected and mock-infected animals, indicating that the pathobiology of *P. gingivalis* in this model is not due to uncontrolled growth of the bacterium or overt damage to *Drosophila* tissues. Our observation that there was no difference in the survival of *Drosophila* infected with live- versus heat-killed strain W83 (Fig. 6) suggests that the pathology caused by the bacterium in this model is due to direct killing of the animals by a *P. gingivalis* component(s) or indirect killing by the host’s own response. Schneider et al. observed that pathogens can kill *Drosophila* via mechanisms that are directly toxic or host mediated (63). The observation that several *P. gingivalis* factors play a role in *Drosophila* killing suggests that killing is multifactorial and not due to a single factor, e.g., a toxin. Host-mediated damage in *Drosophila* can occur either by the hyperactivation of the immune response, or the activation upon infection, of responses that are deleterious to the animals (63). It has been shown that overactivation of the Toll (26) and Imd (8) pathways (major regulators of *Drosophila* immune response genes), excessive production of nitric oxide (10) and reactive oxygen species (32), and the activation of the JNK pathway via Eiger (cytokine) signaling following *Salmonella enterica* serovar Typhimurium (9) or *Mycobacterium marinum* (63) infection results in *Drosophila* lethality. The molecular mechanisms by which these immune responses result in *Drosophila* lethality are currently unknown; however, Schneider et al. and others have suggested that they may be either energetically wasteful or directly toxic (26, 63). *P. gingivalis* major and minor fimbriae, the capsule, and gingipains have been demonstrated to be proinflammatory in mammals (16, 33, 34, 69), and it is known that the bacterium releases outer membrane vesicles (27, 53) that allow pathogen-associated molecular patterns (PAMPs) to be detected at sites distant from the bacterium. As *P. gingivalis* spreads systemically when inoculated into *Drosophila*, PAMPs present in cell surface-associated structures like fimbriae, gingipains, and capsular polysaccharide may induce a systemic hyperactivation of *Drosophila* immune responses, which is deleterious to the bacterium but also harms the animals. Although not systemic, an exaggerated host immune response is primarily responsible for the damage that occurs in periodontitis (61, 67) and in response to *P. gingivalis* infection in other animal models (4, 11, 35).

Despite the bacterium’s inability to multiply effectively in *Drosophila, P. gingivalis* was still capable of killing the animals, and several lines of evidence suggest that *P. gingivalis* killing of *Drosophila* is due to specific interactions between the bacterium and the host. First, *P. gingivalis* (W83)-infected *Drosophila* animals were more likely to die than *E. coli* DH5α-infected *Drosophila* animals (Fig. 1) that received an equivalent inoculum. Second, *P. gingivalis* (W83) killing of *Drosophila* was dose dependent (Fig. 2). Third, strains of *P. gingivalis* exhibited different killing abilities when inoculated into *Drosophila* (Fig. 4). Fourth, several *P. gingivalis* virulence gene mutants are attenuated in the *Drosophila* killing model (Fig. 7). Finally, several immune response-defective *Drosophila* mutants show increased susceptibility to killing by *P. gingivalis* (36).

In this study we have demonstrated that *Drosophila melanogaster* is a viable new animal model for examining *P. gingivalis*-host interactions. The introduction of this highly genetically manipulatable, high-throughput animal model with an innate immune system that is remarkably similar to mammals increases the repertoire of tools available to study *P. gingivalis*-host interactions, especially as the damage that occurs with periodontitis is primarily due to host effects. Although *Drosophila* is not a natural host for *P. gingivalis*, the results of this study suggest that there are many aspects of *P. gingivalis* pathogenesis that are evolutionarily preserved between mammals and *Drosophila*. Therefore, the *Drosophila* killing model could be used to identify *P. gingivalis* factors that are involved in pathogenesis, which is important, as the roles of a large number of *P. gingivalis* genes are unknown. This model could also be used to identify host factors that fight *P. gingivalis* infection or contribute to *P. gingivalis*-induced pathology and to examine the host-pathogen interactions of other oral pathogens. As it is clear that periodontitis is a polymicrobial infection, the *Drosophila* killing model could be exploited to examine the interactions among different oral species and their interactions with host defenses in a mixed bacterial inoculum.

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