# *Drosophila* host defense after oral infection by an entomopathogenic *Pseudomonas* species

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Edited by Kathryn V. Anderson, Sloan–Kettering Institute, New York, NY, and approved June 17, 2005 (received for review March 21, 2005)

***Drosophila*** **has been shown to be a valuable model for the investigation of host–pathogen interactions. Study of the *Drosophila* immune response has been hampered, however, by the lack of true *Drosophila* pathogens. In nearly all studies reported, the bacteria used were directly injected within the body cavity of the insect, bypassing the initial steps of a natural interaction. Here, we report the identification of a previously uncharacterized bacterial species, *Pseudomonas entomophila* (*Pe*), which has the capacity to induce the systemic expression of antimicrobial peptide genes in *Drosophila* after ingestion. In contrast to previously identified bacteria, *Pe* is highly pathogenic to both *Drosophila* larvae and adults, and its persistence in larvae leads to a massive destruction of gut cells. Using this strain, we have analyzed the modulation of the larval transcriptome upon bacterial infection. We found that natural infection by *Pe* induces a dramatic change in larval gene expression. In addition to immunity genes, our study identifies many genes associated with *Pe* pathogenesis that have been previously unreported.**

innate immunity | microarray | host–microbe interaction

*Drosophila* is devoid of an adaptive immune system and relies solely on innate immune reactions for its defense (1, 2). Genetic and molecular approaches have revealed striking similarities between the mechanisms that regulate insect host defense and the mammalian innate immune response. To combat microbial infection, *Drosophila* activates multiple cellular and humoral responses that include melanization, blood coagulation, the production of several effectors such as antimicrobial peptides (AMPs), and the phagocytosis of microorganisms by blood cells. AMPs are made in the fat body, a functional equivalent of the mammalian liver. Genetic analyses have shown that the Toll and Imd pathways regulate AMP gene expression. The Toll pathway plays a critical role in the defense against Gram-positive bacterial and fungal infections, whereas the Imd pathway mediates most responses to Gram-negative bacterial infection. With the exception of the regulation of AMP gene expression, little is known about other defense mechanisms in *Drosophila*. Recently, the use of oligonucleotide microarrays encompassing the full genome has revealed that hundreds of the 13,600 genes are modulated after injection of nonpathogenic bacterial strains into *Drosophila* adults; many of these genes encode antimicrobial peptides, components of signaling pathways, recognition, and effectors molecules (3–5).

To date, most of our knowledge on the *Drosophila* immune response has been built on the analysis of host reactions after direct injection of bacteria into the body cavity of *Drosophila* larvae or adults. Although this approach has been shown to be relevant for identifying pathogen virulence factors and host defense mechanisms, it bypasses the entry of microbes through natural routes of infection (e.g., orally or through the trachea) and subsequent persistence within the organism (6). To overcome these limitations, a natural mode of infection has been developed. This method allowed us to characterize three *Erwinia carotovora* ssp. *carotovora* strains that are able to trigger a strong systemic immune response in *Drosophila* larvae after oral infection (7). Use of one of these strains, *Ecc15*, has been pivotal in revealing not only the ability of *Drosophila* to activate a systemic immune response adapted to the invader but also the induction of local immune responses and the role of NO signaling (7–9). The infectious *Erwinia* strains we previously identified do not have any significant effect on larval viability, indicating that the *Drosophila* defense mechanisms are capable of controlling the infection by eradicating most *Erwinia* cells (7).

Here, we report the identification of a previously uncharacterized bacterial species, *Pseudomonas entomophila* (*Pe*), that can orally infect and kill *Drosophila* larvae and adults. Using microarray, we have compared the transcriptome of larvae infected by either the *Ecc15* strain or by *Pe* together with that obtained after septic injury.

## Materials and Methods

**Insects Stocks.** Oregon$^R$ (Or$^R$) flies were used as a standard wild-type strain. The transgenic strains *Diptericin-lacZ* (*Dpt-lacZ*) and *Drosomycin-gfp* (*Drs-GFP*) were described in ref. 8. A line carrying four copies of the *Dpt-GFP* reporter gene was used to screen the bacterial isolates. *relish* is a recessive mutation that blocks the Imd pathway (10). *Drosophila* stocks were maintained at 25°C.

**Bacterial Strains.** Bacteria were cultured in LB medium with the appropriate antibiotics (100 $\mu$g/ml rifampicin and 600 $\mu$g/ml carbenicilin). The *Ecc15*, *Escherichia coli*, and *Micrococcus luteus* strains were described in ref. 17. The *P. aeruginosa* PA01, *P. putida* KT2440, and *P. syringae* pv. tomato DC3000 strains are referenced in Table 1, which is published as supporting information on the PNAS web site. The other *Pseudomonas* strains tested were from the Collection Française de Bactéries Phytopathogènes and are listed in Table 1. We isolated rifampicin-resistant mutants of *Pe* (*Pe*$^{Rif}$) by using standard procedures. The GFP-expressing strain of *Pe* was generated by introducing plasmid pX2-GFP, which carries the *GFP* gene under the control of a promoter from the *P. aeruginosa* isolate CHA (kindly provided by I. Attree, Centre National de la Recherche Scientifique, Grenoble, France). The *Pe gacA* mutant was obtained from a library of genetic variants generated by random insertion of a *Tn5* derivative (N.V., unpublished data).

**Infection Experiments.** ***Bacterial injection.*** Third-instar larvae were pricked with a thin needle inoculated with a concentrated

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: AMP, antimicrobial peptides; *Pe*, *Pseudomonas entomophila*.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database [accession nos. AY907566 (16S rRNA), AY907568 (*rpoD*), and AY907567 (*gyrB*)].

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bacterial pellet ($4 \times 10^{11}$ cfu/ml) from an overnight culture of *E. coli* and *M. luteus*.

**Drosophila *natural bacterial infection.*** Approximately 200 third-instar larvae were placed in a 2-ml tube containing 200 $\mu$l of concentrated bacterial pellet ($OD_{600} \approx 200$) from an overnight culture and 400 $\mu$l of crushed banana. The larvae, bacteria, and banana were thoroughly mixed in the microfuge tube; the tube was closed with a foam plug, incubated at room temperature for 30 min, and the mixture was then transferred to a standard cornmeal fly medium and incubated at 29°C. Infections for microarray experiments were performed at 25°C to avoid temperature stress. Larvae were collected at different time intervals after infection for $\beta$-galactosidase assays, RT-qPCR analysis (11), microarray analysis, and bacterial counts. For bacterial counting experiments, larvae were first rinsed in water, dipped in 70% ethanol (three times for 5 sec) for external sterilization, and then homogenized and spread onto LB plates containing Rifampicin (100 $\mu$g/ml). *Drosophila* adults were infected as described in ref. 12.

**Bacterial Screening.** Fruit flies or decaying fruits from the Island of Guadeloupe (91 samples) were collected and immediately crushed in 250 $\mu$l of LB medium. Isolates were obtained by plating serial dilution on LB plate. Three different bacterial isolates by sample were then tested for their capacity to induce a systemic expression of *Diptericin* in *Dpt-gfp Drosophila* larvae by using the natural infection procedure described above. *Pe* was isolated from a female *D. melanogaster* collected at Calvaire, Guadeloupe.

**Electronic Microscopy, Phylogeny, Microarrays, and Survival.** For more detailed information, see *Supporting Materials and Methods*, which is published as supporting information on the PNAS web site.

## Results

**Isolation of *Pe*, a Strain That Naturally Infects *Drosophila*.** We collected bacterial isolates from fruit flies and decaying fruits, which provide both a habitat and food for developing larvae, and tested them for their ability to induce a systemic immune response in larvae. Among 91 strains isolated from the western part of the island of Guadeloupe, we found one strain, *Pe*, capable of triggering reproducible *Diptericin* expression in both larvae and adults. We analyzed by RT-qPCR the kinetics of *Diptericin* and *Drosomycin* expression in larvae after natural infection by *Pe* (Fig. 1 *A* and *B*, respectively). Both *Diptericin* and *Drosomycin* transcripts were apparent 3 h after infection and peaked at 24 h in larvae. The level of *Diptericin* expression at 24 h was higher than the level obtained after direct injection of a mixture of Gram-positive (*M. luteus*) and Gram-negative (*E. coli*) bacteria into the body cavity (Fig. 1*A*). In contrast, the *Drosomycin* gene was induced to a lesser extent by *Pe* than by septic injury of the same *M. luteus*/*E. coli* mixture (Fig. 1*B*). These expression patterns indicated that natural infection of *Drosophila* larvae by *Pe* induced a global antimicrobial response with preferential induction of the antibacterial peptide gene *Diptericin* compared with the antifungal peptide gene *Drosomycin*. As observed for *Ecc15* (13), we also observed that the expression of both antimicrobial peptide genes was reduced in *relish* mutant larvae that lack a functional Imd pathway (Fig. 1 *A* and *B*, asterisks).

The larval fat body is located inside the larval haemocoel and is the major site of AMP synthesis. The use of *in vivo Diptericin-lacZ* and *Drosomycin-lacZ* reporter genes demonstrated that natural *Pe* infections strongly induce expression of AMPs in the fat body; almost 90% of the larvae activated a strong systemic immune response (data not shown). In addition, natural *Pe* infections also induced local immune responses: expression of

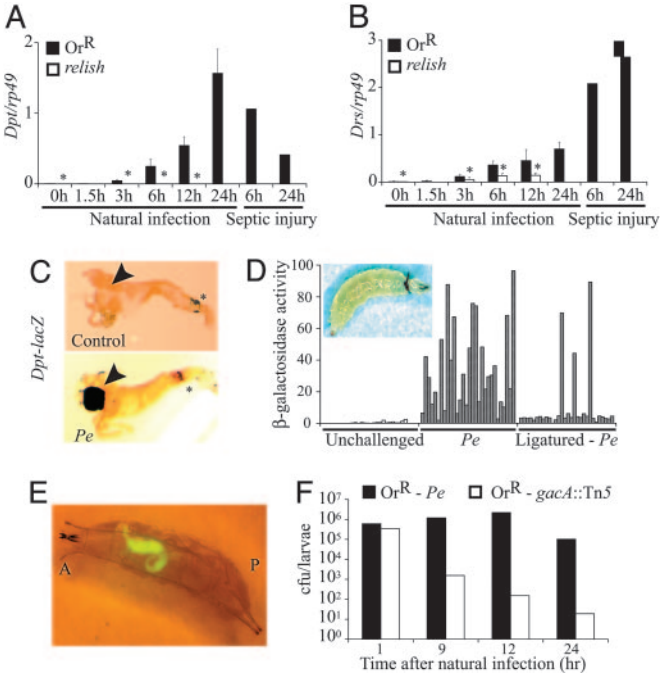


**Fig. 1.** Expression of antimicrobial peptides after *Pe* natural infection and persistence within larval gut. (*A* and *B*) Natural infection by *Pe* induces an Imd-dependent systemic immune response. RT-qPCR analysis shows that *Pe* infection induced sustained *Diptericin* (*A*) and *Drosomycin* (*B*) expression in wild-type larvae ($Or^R$) but not in *relish* mutants (monitored only at 0, 3, 6, and 12 h and indicated by an asterisk). 0 h, unchallenged larvae; *Dpt*, *Diptericin*; *Drs*, *Drosomycin*; *rp49*, *ribosomal protein 49*. For each time point, the values represented are the mean and standard deviation of four and three independent experiments for wild-type and *relish* larvae, respectively. (*C*) *Pe* induced local immune response in the gut: histochemical staining of $\beta$-galactosidase activity is observed in the anterior midgut at the level of the proventriculus (arrowhead) of wild-type infected larvae that carry the *Dpt-lacZ* reporter gene. Larvae were collected 24 h after infection. *, endogenous $\beta$-galactosidase activity. Similar results were obtained with a *Dpt-GFP* reporter gene (data not shown). (*D*) *Dpt-lacZ* larvae that were sealed at the mouth with a strand of human hair (*Inset*) were naturally infected by *Pe* and collected at 12 h. Each bar represents the level of $\beta$-galactosidase activity measured in a single larva. Ligatured larvae generally failed to express *Dpt-lacZ* after exposure to *Pe*, demonstrating that the digestive tract is the major route of infection for *Pe*. Unchallenged, untreated larvae; natural infection, larvae infected by *Pe*; natural infection ligature, ligatured larvae infected by *Pe*. (*E*) GFP expressing *Pe* are observed in the anterior part of the midgut in infected larvae 6 h after infection. A, anterior; P posterior. (*F*) Bacterial persistence was measured in wild-type ($Or^R$). Bacterial counts were obtained by plating the larval homogenates of five surface-sterilized larvae that were naturally infected with a rifampicin-resistant strain of *Pe* and its *gacA*::Tn*5* derivative on LB medium containing rifampicin (100 $\mu$g/ml). The number of colony-forming units (cfu) per larva obtained at each time point after infection represents the mean of three independent measurements.

*Diptericin* was detected in the anterior midgut at the level of the proventriculus (Fig. 1*C*), whereas *Drosomycin* expression was detected in the trachea (data not shown). To determine the route of infection, we monitored *Diptericin-lacZ* reporter gene expression in individual larvae sealed at the mouth with a strand of human hair before *Pe* exposure (Fig. 1*D*). The level of $\beta$-galactosidase activity was very much reduced in ligatured larvae compared with control larvae, indicating that the digestive tract is the main route of infection. The use of a fluorescent *Pe* obtained by expressing the GFP constitutively also revealed bacterial accumulation in the digestive tract, most frequently in the anterior midgut (Fig. 1*E*). In contrast to *Ecc15*, we also observed that *Pe* triggers antimicrobial peptide gene expression

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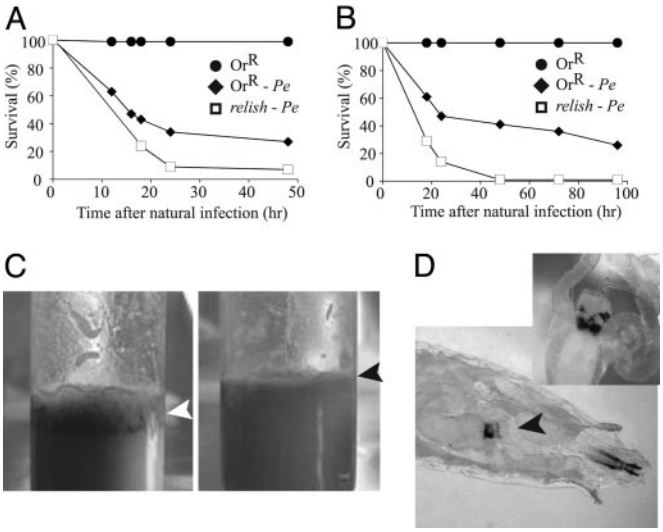


**Fig. 2.** Natural infection by *Pe* kills both *Drosophila* larvae and adults. (*A*) Wild-type ($Or^R$) and *relish* larvae were naturally infected by *Pe*. *Pe* infections kill 70% of wild-type larvae within 48 h. *relish* larvae were more susceptible to *Pe* infection (90% lethality at 48 h) compared with $Or^R$ larvae. (*B*) Wild-type ($Or^R$) and *relish* adult flies were naturally infected by *Pe*. *Pe* infection kills 70% of wild-type adults within 4 days. *relish* flies were more susceptible than wild-type to *Pe* infection. Unchallenged *relish* larva and flies survived as wild type (data not shown). (*C*) *Pe* ingestion induces food uptake blockage. The medium of unchallenged larvae is kneaded (*Left*, open arrowhead) contrary to what observed in infected larvae (*Right*, filled arrowhead). (*D*) Infection by *Pe* often induces melanization at the level of the surface of the proventriculus visible in living larvae or on dissected gut (*Inset*).

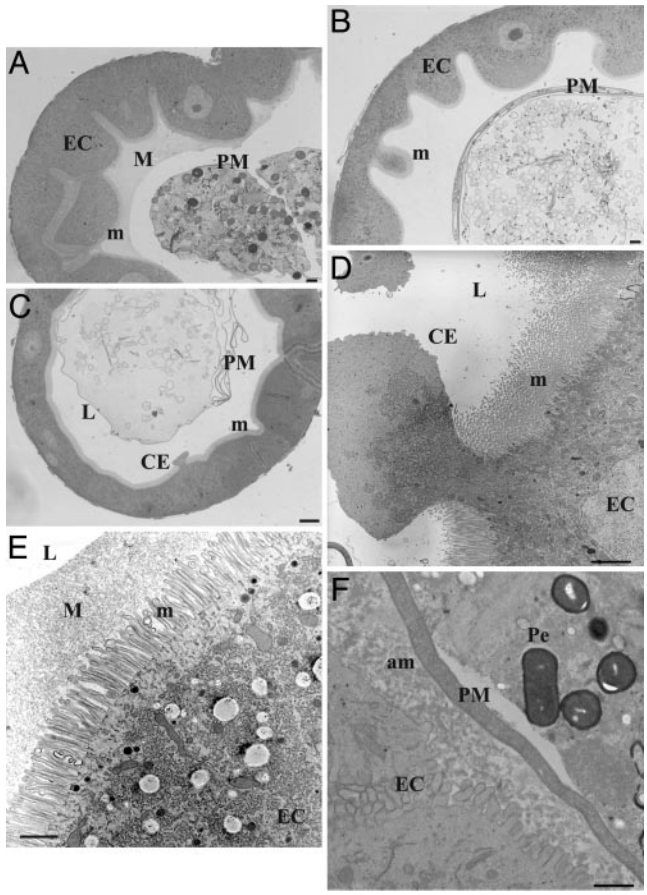


**Fig. 3.** *Pe* infection provokes a strong perturbation of the *Drosophila* larval midgut. Transversal sections of larval anterior midgut collected at 6 h (*A–D*) or 12 h (*E* and *F*) after natural infection by *Pe* (*B–D* and *F*) or a *gacA*::Tn*5 Pe* avirulent derivative (*A* and *E*) were analyzed. (*A–C*) Semithin sections were observed under bright field. (*D–F*) Ultra-thin sections were observed by transmission electron microscopy. At 6 h after *Pe* infection, the mucus that protects the digestive epithelium was absent (compare *B* with *A*), and the gut cells showed extrusion of cell materials into the lumen (*C* and *D*). At 12 h after infection, the cells display abnormal microvilli (compare *F* with *E*). M, mucus; L, lumen; m, microvilli; PM, peritrophic matrix; *Pe*, *P. entomophila*; CE, extrusion of cell materials; am, abnormal microvilli; EC, epithelial cell. (Scale bar: *D–F*, 1 $\mu$m; *A–C*, 5 $\mu$m.)

in adults although the response was less reproducible than in larvae (data not shown).

***Pe* Is Highly Pathogenic to *Drosophila*.** In contrast to *Ecc15*, we observed that *Pe* was highly pathogenic to both *Drosophila* larvae and adults (Fig. 2 *A* and *B*). Oral infection by *Pe* induced >70% mortality in larvae within 24 h and the remaining 30% of animals died at the pupal stage. Interestingly, both larvae and adults mutated in the *relish* gene succumbed faster, indicating a contribution of the Imd pathway in the defense against this bacterium. Infected larvae were usually smaller than control larvae and the medium of the tube into which they were transferred was not kneaded suggesting that *Pe* infection provoked food uptake blockage (Fig. 2*C*). At late time points, *Pe* infection induced melanization spots on the gut surface in 30% of living larvae (Fig. 2*D*), which suggests damage of the gut. To further investigate the impact of the infection on gut morphology, we performed histological analyses at different time points. Electron and optic micrographs of transversal sections of larval midgut indicated that *Pe* infection provoked a strong perturbation of the midgut epithelium (Fig. 3). At 6 h after infection, the mucus that protects the digestive epithelium was absent in *Pe*-infected larvae (Fig. 3*B*) compared with the control (Fig. 3*A*). Interestingly, some gut cells showed extrusion of cellular material into the lumen, the cytoplasm extruded from the cells still being delimited by the plasma membrane (Fig. 3 *C* and *D*). The modifications caused by *Pe* were more dramatic at 12 h after infection. Epithelial cells disappeared or displayed abnormal microvilli compared with noninfected larvae (Fig. 3 *E* and *F*). In the gut, the epithelium is lined by a semipermeable matrix called the peritrophic matrix. This barrier acts as an efficient filter, allowing the passage of small molecules such as nutrients while preventing that of bigger objects such as microorganisms. In nearly all cases, we observed that this peritrophic matrix was present. To determine whether *Pe* was able to cross the gut, we analyed the presence of *Pe* in the hemolymph of infected larvae by bacterial count; no bacteria were detected at 6 h after infection. This result indicates that *Pe* infection did not lead to a major invasion of the haemocoel, and if bacteria crossed the gut at early time points, they did not persist in the hemolymph.

***Pe* Is a Previously Uncharacterized *Pseudomonas* Species.** The comparison of *Pe* 16S rRNA sequence with 16S rRNA sequences present in the database clearly indicated that *Pe* belongs to the *Pseudomonas* genus. The three dendrograms deduced from coding (*rpoD* and *gyrB*) and noncoding (16S rRNA) sequences showed that *Pe* is closely related to *P. putida*, a metabolically versatile saprophytic soil bacterium (Fig. 6, which is published as supporting information on the PNAS web site, for 16S rRNA-based phylogenetic tree and data not shown). The 16S rRNA-based dendrogram allowed us to show that, within the *P. putida* group, *Pe* was found closely related to *P. monteilii* and *P. mosselii*, two poorly characterized species. Because *Pe* is a previously uncharacterized *Pseudomonas* species with some unique ento-

mopathogenic properties, we designated it *Pseudomonas entomophila* (*Pe*).

The extraordinary versatility of *Pseudomonas* species is reflected by their ability to colonize numerous ecological niches. As a consequence, they are virulent toward plants, insects, and nematodes and are a major cause of human opportunistic infections (14, 15). It is well established that *P. aeruginosa* is pathogenic to flies when injected in the body cavity (12, 16–19). *Pe* is however the first bacterium of this genus to be highly pathogenic for both *Drosophila* larvae and adults by oral ingestion. To test the specificity of the *Pe*/*Drosophila* interaction, we analyzed 28 strains that cover the *Pseudomonas* genus for their ability to infect *Drosophila*. Of these 28 strains, only four, other than *Pe*, induced weak *Diptericin* expression, whereas the others were noninfectious (Table 1). None of the 28 *Pseudomonas* strains tested induced significant lethality to larvae or adults (Table 1 and data not shown), indicating that the relationship between *Drosophila* and *Pe* is highly specific, and the lethality provoked does not result from the general metabolic properties of *Pseudomonas* species

Together with *gacS*, *gacA* encodes the GacS-GacA two-component system involved in the control of multiple processes, including virulence in *Pseudomonas* species (20). We constructed a *Pe* derivative carrying a Tn*5* minitransposon in the *gacA* gene (*gacA*::Tn*5*; N.V., unpublished data). Interestingly, the *gacA*::Tn*5* mutant failed to activate a strong immune response after feeding and did not exhibit any pathogenicity toward larvae or adults (data not shown), indicating that *Pe* virulence is under the control of GacA-GacS two component system. We compared gut persistence of wild-type *Pe* and the *gacA*::Tn*5* mutant by quantifying the number of bacteria in larvae and adults at different time points after infection. Whereas *gacA*::Tn*5* bacterial levels decreased rapidly with time, *Pe* titer remained high indicating survival and persistence inside the host (Fig. 1*F*).

***Pe* Induce Most Larval Immune-Regulated Genes.** To date, no study has analyzed the modulation of the transcriptome of *Drosophila* larvae after oral infection by Gram-negative bacteria. The isolation of *Pe*, together with availability of *Ecc15*, offers a unique opportunity to compare the set of genes induced in larvae by a pathogenic Gram-negative bacterium to those induced by nonpathogenic bacteria. We subjected larvae to oral infection with the infectious but nonpathogenic strain *Ecc15*, the infectious and pathogenic strain *Pe*, and its noninfectious and nonpathogenic isogenic derivative *gacA*::Tn*5* or to septic injury by a mixture of Gram-negative (*E. coli*) and Gram-positive (*M. luteus*) bacteria. Septic injury was used to determine the larval genes modulated during a systemic response. Total RNA from whole larvae collected 0, 1.5, 3, 6, and 12 h after infection were hybridized to Affymetrix DrosGenome1 GeneChips. The gene expression profiles obtained were normalized to unchallenged larvae collected at the same time point to eliminate genes whose expression is modulated during larval development independently from infection. Among the 13,600 genes present on the arrays, 436 were selected for a 2-fold change in any of the time points compared with the unchallenged kinetic in all infection procedures. Comparison of the four sets of data (*Pe*, *Pe gacA*, *Ecc15*, and septic injury) allowed us to classify the genes into different categories (Fig. 4). Fig. 5 shows a short list of selected genes (see Tables 2–5, which are published as supporting information on the PNAS web site, for the complete list).

This analysis allowed us to identify 99 genes whose expression varied in response to septic injury (Table 2). Many of these genes are likely to be induced by the fat body and reflect the larval systemic immune response. Among them, 27 genes were exclusively induced upon septic injury (Fig. 5 and Table 2). The presence of genes encoding serine proteases, serpins, and components of the melanization cascade in this category points to their role in the wound-healing reaction associated with the physical injury. This class also includes *PGRP-SA* that encodes a recognition protein required to combat Gram-positive bacterial infection (21).

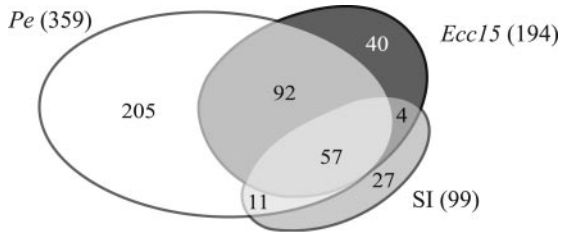


**Fig. 4.** General statistics on the larval genes regulated after natural infection and septic injury. The graph shows the number of genes induced or repressed after natural infection by *Pe* (white), *Ecc15* (black) and after septic injury with a mixture of *E. coli* and *M. luteus* (SI, gray). The 57 genes modulated by *Pe*, *Ecc15*, and septic injury belong to the core of immune responsive gene. The 92 genes modulated by both *Ecc15* and *Pe* but not septic injury constitute the core of natural infection specific genes. The 27 genes modulated only by the septic injury may play a role in wound healing and response to Gram-positive bacterial infection.

Importantly, we observed that oral infection by *Ecc15* and/or *Pe* induced 72 of the 99 genes induced by septic injury (Fig. 4). This result indicates that naturally infectious Gram-negative bacteria are able to induce most of the larval immune-regulated genes. Among these genes are 11 antimicrobial peptide genes, 3 genes involved in recognition and phagocytosis, 6 encoding small immune-regulated peptides, and 21 unknown genes. All of these genes display more rapid induction kinetics after septic injury than after oral infection, even if their maximal expression is similar (e.g., AMPs in Fig. 5), thus reflecting a delay in the activation of immune signaling cascades when bacteria are orally transmitted.

**The Core Response to Gram-Negative Bacteria Oral Infection.** Oral infection by both *Ecc15* and *Pe* specifically affects the expression of a subset of 92 genes that were found induced or repressed in both natural infections but not upon septic injury (Fig. 5 and Table 3). Among them, 15 genes encode proteins involved in general metabolism, 5 encode peritrophic matrix constituents, 1 lectin, and 39 genes of unknown function. It is worth mentioning that genes encoding a protease (CG15255) and four proteins of unknown function (CG6640, CG16775, CG14499, and CG13482) are up-regulated by >8-fold after infection by *Ecc15* or *Pe*. Only 30 genes were also modulated after oral infection by the *gacA*::Tn*5* mutant (Tables 2 and 3). Among these genes are AMPs that displayed a significantly lower induction than in *Pe* or *Ecc15* infections (Table 2). This finding is in agreement with the observation that <10% of the larvae infected by *gacA*::Tn*5* induce a systemic immune response (see above).

***Pe* Induces Strong Changes in Expression Profile.** In addition to the genes whose expression is modulated by both *Pe* and *Ecc15* infections, *Pe* infection specifically modifies the expression of a further 205 genes (Fig. 5 and Table 4). Interestingly, >90% of these genes are up-regulated only 12 h after *Pe* infection and are likely to be associated with the alteration of the gut physiology observed. Among these *Pe*-specific genes, more than half can be assigned to six functional groups (Table 4): (*i*) serine protease inhibitors (7 genes including *Spn4* and *Spn6*), (*ii*) detoxification and stress response [14 genes including 6 GSTs (*GST24D* >10-fold), 4 cytochromes (*cyp12c1* >10-fold), 1 oxidase (CG18522 involved in the response to reactive oxygen species) and 3 others], (*iii*) general metabolism (33 genes), (*iv*) transcrip-

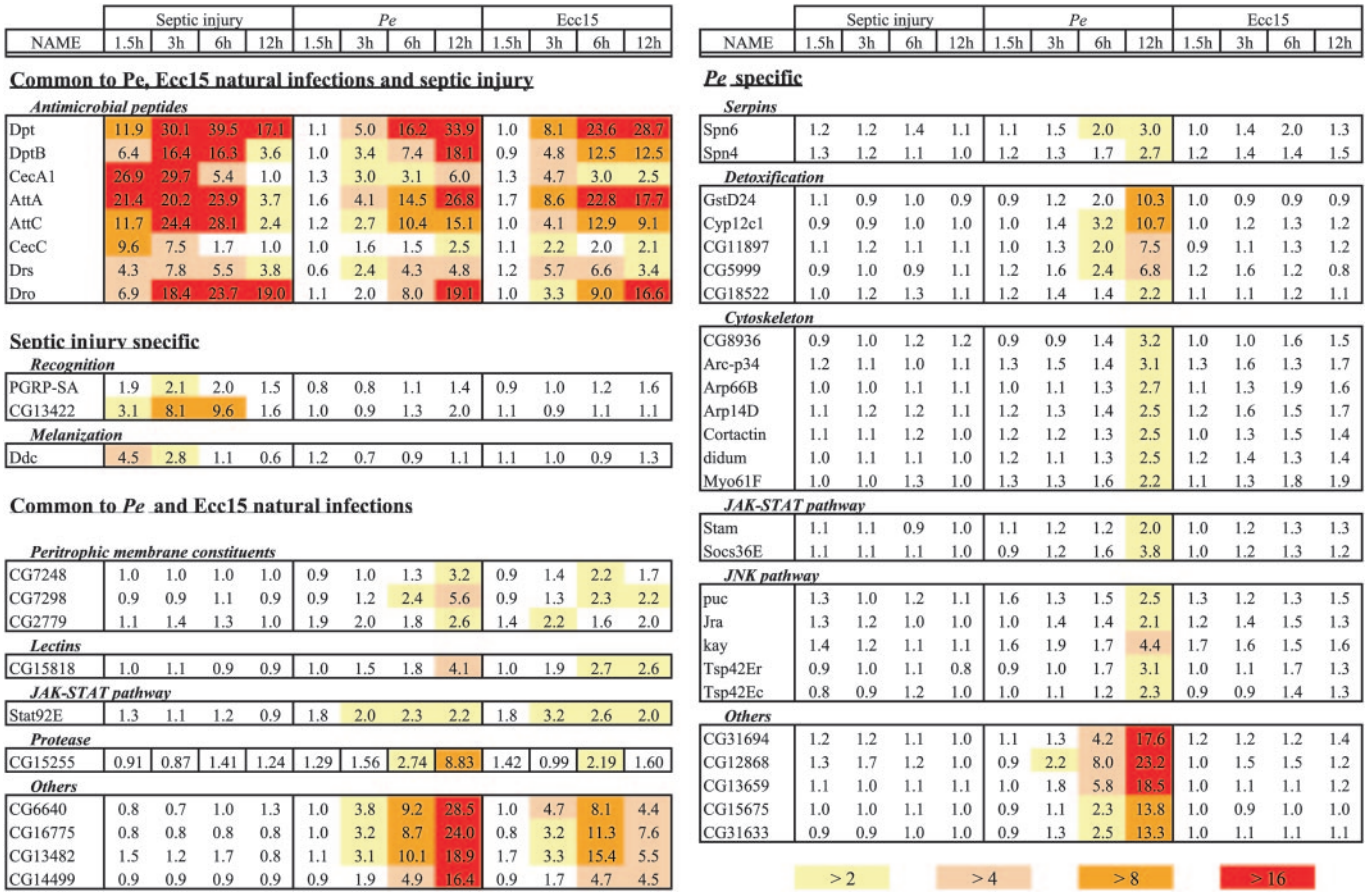


**Fig. 5.** Examples of genes regulated by the different types of immune challenge. The fold change after septic injury and natural infection compared with uninfected larvae for selected genes is shown. Time intervals after infection are indicated in hours on the top. The GO IDs are references to entries in the GENEONTOLOGY index of molecular functions and biological processes (www.geneontology.org). For simplicity, the values for the *gacA*::Tn*5* infection have been removed. The fold change color code is indicated at the bottom right.

tion and protein processing (17 genes), (*v*) cytoskeleton (16 genes including actin cytoskeleton, *Cortactin*; Arp2/3 complex, *Arp66B* and *Arp14D*; and myosin cytoskeleton, *didum* and *Myo61D*), and (*vi*) signal transduction (44 genes). Among this latter group are found proteins associated with the JAK/STAT pathway (*Stam*, *Socs36E*, *STAT92E*, and CG15154) and the JNK pathway (*puc*, *Jra*, and *kay*). The up-regulation of genes encoding components of the JNK pathway and known targets of this pathway such as *puc*, *Myo61D*, *Tsp42E* (*Tsp42Ec* and *Tsp42Er*), and *scb* (22) clearly indicates that *Pe* infection triggers the JNK pathway. *Pe* infection also regulates the expression of 22 genes unassigned to any of these functional groups of which CG31694, which carries an uncharacterized hydrolase activity, is up-regulated >16 fold and 60 genes of unknown function of which 5 (CG12868, CG13659, CG15675, CG31633, and CG11825) are up-regulated by >10 fold.

In sharp contrast, genes specifically induced by *Ecc15* infection include 40 genes (Table 5), none of them being induced by >3-fold. Among the *Ecc15*-specific genes is *PGRP-SC1*, which encodes a secreted amidase known to be strongly expressed in the gut (23).

## Discussion

In this study, we have identified a bacterium, *Pe*, collected from a fly isolated in Guadeloupe that induces a strong systemic immune response and kills *Drosophila* larvae and adults after oral infection. The analysis of larvae infected with a set of 28 *Pseudomonas* strains revealed that the interaction between *Pe* and *Drosophila* was highly specific. Histological analyses of infected larvae throughout the course of the infection indicated that *Pe* induced a strong perturbation of the gut physiology. Interestingly, we observed that *Pe* is able to kill larvae of several other insect species, indicating that *Pe* is a previously uncharacterized entomopathogenic species with a potentially wide host range (P.L., unpublished data). The mechanisms by which *Pe* kills flies remains to be investigated but we cannot exclude at this stage that *Pe* virulence is mediated by a toxin, as described in other entomopathogenic bacteria such as *Photorhabdus luminescens* or *Bacillus thuringiensis* (24). Interestingly, none of these bacterial strains were pathogenic to *Drosophila* larvae after oral ingestion with our assay (7) (unpublished data). This finding demonstrates that *Pe* has some specific entomopathogenic properties that might be used as a source for developing novel biopesticides. The observation that *Pe* virulence depends on the GacA-GacS two-component system, however, suggests that *Pe* shares with other *Pseudomonas* species similar strategies for the regulation of virulence factors.

Recently, it has been proposed that the Toll and Imd pathways may play a role against saprophytes rather than pathogenic microbes (2). Our observation that both larvae and adults mutated in *relish* succumbed faster than wild type to *Pe* infection demonstrates a contribution of the Imd pathway in the resistance against orally transmitted pathogens. Other defense mechanisms may play an important role against *Pe* infection. Interestingly, the microarray analysis reported here indicates that several genes encoding peritrophic matrix constituents are up-regulated after

*Pe* infection. The reinforcement of this physical barrier may play a defensive role by preventing contact between the bacteria and the gut epithelium. The observation that *Pe* induces many genes involved in oxidative stress and/or detoxification such as GSTs, oxidase, and cytochromes is consistent with a recent study indicating that the homeostasis of redox balance is one of the critical factors affecting host survival during continuous host–microbe interaction in the gastrointestinal tract of *Drosophila* (25). However, the high mortality observed suggests that *Pe* can effectively overcome the *Drosophila* host defense.

Analyses of the *Drosophila* transcriptome after immune challenge led the way to several successful postgenomic studies uncovering previously uncharacterized aspects of the fly immune system. This report extends previous studies by analyzing the larval transcriptome after septic injury and oral bacterial infection. Surprisingly, we noted that septic injury induced fewer genes in larvae than in adults. For instance, genes encoding lipases, a peroxidase, Idgfs, a high number of serine proteases, and serpins that were found up-regulated in adults (3) were not induced in larvae after septic injury. This result indicates that the repertoire of immune genes regulated at the transcriptional level is lower in larvae compared with adults. The microarray analysis shows that oral infection by *Pe* induces most of the genes up-regulated after a systemic infection. Analysis of *Diptericin* and *Drosomycin* expression strongly suggests that the fat body response depends on the Imd pathway in agreement with its function in the response to Gram-negative bacterial infection. Our study also demonstrates the complexity of the larval immune response after oral infection with Gram-negative bacteria by revealing a large subset of genes found only in larvae infected by *Ecc15* or *Pe*. Most of these genes might constitute the host response to bacterial persistence in the gut. The presence of genes encoding proteases, constituents of the peritrophic matrix and others involved in general metabolism, may reflect the physiological modifications that the gut cells undergo because of the presence of these bacteria. *Pe* infection also triggers many genes encoding cytoskeleton components that are regulated by the JNK pathway reflecting the major modification of the gut epithelium. Among all of these genes were a few that were induced by >6-fold. These genes might constitute promising postgenomic targets for a more detailed understanding of the infection process. Altogether, our results suggest that *Ecc15* or *Pe* similarly induce a systemic immune response after being ingested. It seems that the bacterial persistence in the first loop of the larval midgut is a key step in the activation of a systemic immune response. In *Ecc15*, this persistence is promoted by a single gene, *evf* (26), that appears to be absent from the *Pe* genome (data not shown). These results indicate that these two bacteria have developed independent strategies to persist within the gut of their host. A major issue will be the identification of the mechanisms that link bacterial persistence in the gut to the induction of a systemic immune response in the fat body. It remains to be determined whether the systemic immune response is caused by an early crossing of the gut by *Pe*.

In this study, we have identified an oral entomopathogenic bacterial species and determined the corresponding host response in *Drosophila* by using microarray analysis. The availability of genetic tools for *Pseudomonas* bacteria combined with those of *Drosophila* provides a unique model for the dissection of host–pathogen interactions. The *Drosophila*/*Pe* interaction may give more insight into *Pseudomonas* pathogenesis and into the physiology of the *Drosophila* immune response. In contrast to the previously identified *Ecc15* strain, *Pe* should allow the study of *Drosophila* pathogenesis in both larvae and adults.

We thank H. Roberta Koepfer and Peter C. Chabora (Queens College, City University of New York, New York), who collected the bacterial samples in Guadeloupe; Dr. Louis Gardan for providing *Pseudomonas* species; Dr. Ina Attree for providing *Pseudomonas* genetic tools and advice; Brigitte Maroni, Hélène Labit, Gaëlle Demarre, Stéphane Chiron, Danielle Jaillard, and Garson Tsang for technical support; and Mark Blight and Christopher Scherfer for critical reading. Paul Spellman performed this work when he was a postdoctoral fellow in the laboratory of Gerald Rubin, and we thank Gerald Rubin for his support and encouragement. This work was supported by the Bettencourt Foundation, the Schlumberger Foundation, and the Program Microbiologie (PRMMIP00). N.V. is supported by a doctoral fellowship from the Association Vaincre La Mucoviscidose and the Association de Recherche sur le Cancer.

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