Cyanide Produced by Human Isolates of Pseudomonas aeruginosa Contributes to Lethality in Drosophila melanogaster

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Some Pseudomonas aeruginosa strains are cyanogenic, and cyanide may contribute to the bacterium’s virulence. Using human isolates of P. aeruginosa, we have shown that Drosophila melanogaster suspended above cyanogenic strains become motionless and develop bradycardia and that flies injected with cyanogenic bacterial strains die more rapidly than those injected with noncyanogenic strains. Flies exposed to cyanogenic strains had high cyanide and low adenosine triphosphate (ATP) concentrations in body extracts, and treatment with a cyanide antidote equalized survival of flies injected with cyanogenic and noncyanogenic strains. P. aeruginosa PAO1 strain with a mutation in the hydrogen cyanide synthase gene cluster was much less toxic to flies than the parental cyanogenic strain or 2 knock-in strains. Transgenic flies overexpressing rhodanese, which detoxifies cyanide by converting it to thiocyanate, were resistant to cyanide and the increased virulence of cyanogenic strains. We conclude that D. melanogaster is a good model for studying cyanide produced by P. aeruginosa.

METHODS

Materials. P. aeruginosa isolates from 20 randomly selected patients at the University of California, San Diego,
Medical Center were obtained without patient identifiers; 4 isolates were from patients with cystic fibrosis, and all were confirmed as *P. aeruginosa* by standard biochemical tests. *P. aeruginosa* strain PAO1 was used in some studies because of the availability of a hcnC (hydrogen cyanide synthase) mutant strain and the mutant strain with the hcnABC gene cluster reinserted [11]. *Escherichia coli* strain DH-5α was from Invitrogen. Canton-S wild-type *D. melanogaster* were used in all studies, except the w^1118^ strain was used to generate flies overexpressing rhodanese. Cobinamide was prepared from hydroxocobalamin [16].

**Measurement of bacterial cyanide production.** Bacteria were plated on Luria-Bertani agar in 8 × 9-cm (height × width) screw-cap glass jars and grown overnight to a confluent lawn containing approximately equal numbers of bacteria per jar. The caps were removed for 10 min to equilibrate with air, and the jars were recapped. After 1 h at 37°C, 10 mL of gas was withdrawn from the jar via an airtight port into a gas-tight syringe containing 1 mL of 100 mmol/L NaOH. The syringes were shaken for 1 h at room temperature to convert HCN to sodium cyanide; the latter was measured spectrophotometrically after a color change of p-nitrobenzaldehyde and o-dinitrobenzene [14, 17].

**Measurement of bacterial thiocyanate production.** Bacteria were grown in Luria-Bertani broth to an OD of 0.5–1.0 and were removed by centrifugation. In a capped tube, thiocyanate in the broth was oxidized to cyanide using acidified potassium permanganate and collected in 100 mmol/L NaOH in a well suspended above the broth [18]. The resulting sodium cyanide was measured as described above.

**Exposure of flies to bacteria and to cyanide gas.** Flies were placed into 13 × 50-mm glass cylinders stoppered with glass wool at both ends [14, 16]. The cylinders were taped to the wall of jars ~5 cm above a bacterial lawn (generated as described above), assuring no contact between the flies and bacteria. The jars were kept at 37°C, and fly activity was observed every 10 min, with any movement scored as active. After 100 min, the glass cylinder was removed from the jar and placed in ambient air, and the flies were observed for an additional 150 min.

Flies were exposed to cyanide gas in glass vials by spotting potassium cyanide onto filter paper in the vial; the paper contained sufficient acid to generate HCN, and the resulting cyanide gas concentration was ~22 ppm [14]. Under these conditions, all flies fall motionless within 15 s; they were removed from the vial after 1 min and observed for 1 h.

**Measurement of the cyanide content of flies.** The cyanide content of flies was measured by decapitating the flies (to eliminate interference of eye pigments) and extracting the bodies in 1 mmol/L NaOH [14]. The resulting sodium cyanide was measured spectrophotometrically as described above.

**Assessment of *D. melanogaster* heart rate.** *Drosophila* heart rates were measured by microscopic image analysis [19, 20]. Briefly, flies exposed to a lawn of *P. aeruginosa* were transferred to a microscope slide, and a moving image of each fly heart was recorded. A 2-dimensional time-space (M mode) representation of cardiac contraction was generated from image intensities measured along a line perpendicular to the heart tube. The frequency of contraction was derived from the average intensity of pixels on the line, using a beat-detection algorithm [19, 21].

**Injection of flies with bacteria.** Flies were anesthetized on ice, and 1 μL of bacterial suspension containing ~5 × 10^6^ bacteria was injected into the flies’ thoraxes [20]. The number of live flies were counted during the following 48 h. In some experiments, cobinamide, hydroxocobalamin, or sodium thiosulfate was added to the bacterial suspension at concentrations of 500, 500, and 100 μmol/L, respectively. Because the fluid volume of a fly is ~10 μL [22], the final concentration of the drugs was 10-fold less.

**Measurement of nitrite and nitrate in *D. melanogaster*.** Nitric oxide (NO) has a very short half-life and is rapidly oxidized to nitrite and nitrate. We assessed NO production by measuring nitrite and nitrate in fly extracts by means of an enhanced Griess reagent [16, 20, 23].

**Measurement of ATP in *D. melanogaster*.** Flies were injected with bacteria as described above; 3 h later they were decapitated, and their bodies were extracted in acid. ATP was measured in neutralized extracts by means of firefly luciferase [24].

**Generation of *D. melanogaster* overexpressing bovine rhodanese.** The full-length bovine adrenal rhodanese gene was excised from WT-Rho/pET11d [25] and cloned into the pCaSpeR 4 *Drosophila* expression vector (from J. Radford, Burnham Institute). Purified DNA was injected into 200 *Drosophila* embryos of a w^1118^ host line (BestGene). Eight stable lines were obtained; WT-Rho expression was assessed by reverse-transcription polymerase chain reaction (RT-PCR) on mRNA extracted from wild-type and transgenic flies. The primer sequences for bovine rhodanese were 5'-GACATAGAGGAGTGTCGGGAC-3' (forward) and 5'-GATGGCTGCTCTGGCCGTGA-3' (reverse). The RT-PCR product was assessed by agarose gel electrophoresis.

**Data analysis.** Data presented in bar and line graphs are the means ± SDs of at least 3 independent experiments performed in duplicate or on the number of flies stated in the figure legends. Differences between groups were analyzed by a 1-way analysis of variance with either a Dunnett posttest comparison with the control group or a Bonferroni comparison of selected groups; differences for which *P* < .05 were considered significant.

**RESULTS**

**Effects of Cyanide Produced by *P. aeruginosa* on *D. melanogaster*.**

**Cyanide production by *P. aeruginosa*.** *P. aeruginosa* isolated from 20 different patients produced varying amounts of cyanide over approximately a 15-fold range. Of the 20 isolates, 3 produced high, 10 produced intermediate, and 7 produced low
amounts of cyanide, defined as >10, 1–10, and <1 nmol/min/ confluent bacterial lawn, respectively, in a 9-cm-diameter jar. We also measured thiocyanate produced by the bacteria and found that it correlated reasonably well with cyanide production \( (r^2 = 0.67) \), suggesting that differences among the strains was due largely to cyanide generation and not to cyanide conversion to thiocyanate by bacterial rhodanese.

We chose 1 low-, 1 intermediate-, and 1 high-cyanide-producing isolate for further study, referring to these isolates as strains A, B, and C, respectively. Strain A likely did not produce any cyanide, because it yielded cyanide production values similar to those in jars containing agar only or Escherichia coli (figure 1A; the agar-only jar can be considered the assay background). Strains B and C produced cyanide at 6.1 ± 1.3 and 10 ± 1.9 nmol/min/confuent bacterial lawn, respectively (figure 1A).

**Fly activity after exposure to P. aeruginosa.** Exposing D. melanogaster to the 3 P. aeruginosa strains yielded very different results. After 100 min, flies exposed to strain A exhibited normal activity, whereas almost all flies exposed to strain C were inactive, and only ~40% of flies exposed to strain B were active (figure 1B, white symbols). The flies had no direct contact with the bacteria but were in a small cylinder suspended ~5 cm above the bacterial lawn. These data suggest that a volatile compound released by the bacteria inhibited fly activity, and, because the potency of the effect correlated with cyanide production by the 3 strains, it seemed possible that cyanide was the responsible agent. As further evidence that cyanide caused the reduced fly activity, flies grown on food containing the cyanide scavenger cobinamide showed significant resistance to the inhibitory effect (figure 1B, black symbols).

We demonstrated previously that cyanide inhibition of cytochrome-c oxidase is reversible in vivo [14], and we found that flies exposed to strains B and C slowly recovered activity after being removed from the jars; recovery of flies exposed to strain B was complete by 150 min, and ~60% of the flies exposed to strain C had recovered by that time (figure 1C). Flies exposed to strain A remained fully active throughout the 150-min interval.

**Fly heart rates after exposure to P. aeruginosa.** Because the mammalian heart and brain rely on high amounts of oxygen, they are considered to be primary targets of cyanide [5, 6]. We therefore assessed the effect on fly heart rates of exposure to the 3 Pseudomonas strains. Flies exposed to strain A had heart rates similar to those in flies exposed to agar alone (figure 1D) and similar to rates we had found previously in normal flies [20]. Flies exposed to strains B and C had significantly lower heart rates than those exposed to agar or strain A (figure 1D). The decreased heart rates in flies exposed to strains B and C were likely from cyanide, because exposing flies to 22 ppm of cyanide gas (a lethal cyanide dose for flies [14]) reduced heart rates to below our limits of detection.

**Cyanide content of flies.** If cyanide produced by the bacteria was responsible for inhibiting fly activity and cardiac function, then cyanide levels should be elevated in more severely affected flies. We found similar cyanide levels in flies exposed to P. aeruginosa strain A and in flies exposed to agar or E. coli, levels that may result from cyanogenic substances in fly food, but markedly elevated cyanide levels in flies exposed to P. aeruginosa strain C and moderately elevated cyanide levels in flies exposed to strain B (figure 1E). Under all conditions, flies grown on cobinamide-containing food had significantly lower cyanide levels (figure 1E, black bars).

**Contribution of Cyanide Produced by P. aeruginosa to Lethality in D. melanogaster**

Because of the lethal effects of cyanide, flies infected with cyanide-producing strains of P. aeruginosa should exhibit higher death rates than flies infected with non–cyanide-producing strains. Consistent with this hypothesis, injecting strain C into flies killed all the flies within 24 h, whereas injecting strains B and A killed ~80% and 55% of flies, respectively (figure 2A, white symbols). Half the flies were dead after ~5 h for strain C, 15 h for strain B, and 33 h for strain A. The difference in lethality among the 3 strains appeared to be from cyanide, because flies grown on cobinamide and injected with strains B and C had death rates similar to those in flies injected with strain A (figure 2A, black symbols). Two other cyanide-detoxifying agents, hydroxocobalamin and sodium thiosulfate, also reduced mortality in bacteria-injected flies, although neither of these agents was as effective as cobinamide (figure 2B; data shown for strain C only).

**Effect of P. aeruginosa on Nitrite, Nitrate, and ATP Concentrations in D. melanogaster**

Bacterial sepsis is associated with vasopressor-refractory hypotension secondary to high NO production by endotoxin induction of type 2 NO synthase (iNOS) [26]. We showed previously that injecting D. melanogaster with E. coli or Staphylococcus aureus increases nitrite and nitrate levels several fold and that cobinamide, which binds NO in addition to cyanide, prevents the increase in nitrite and nitrate [20]. To determine whether some of the difference in toxicity among P. aeruginosa strains A, B, and C could be secondary to differences in iNOS stimulation, we measured nitrite and nitrate 2 h after injecting flies with bacteria. We found no significant differences in nitrite and nitrate levels in flies injected with the 3 different P. aeruginosa strains, which suggests that bacterial stimulation of NO production did not account for differences in toxicity among the 3 strains (figure 3A). As in the previous studies with E. coli and S. aureus, feeding flies cobinamide prevented the subsequent increase in nitrite and nitrate (figure 3A, black bars).

Because cyanide is a potent inhibitor of cytochrome-c oxidase, cyanide-producing strains of P. aeruginosa should decrease ATP concentrations in bacterially infected flies. We found that inject-
Figure 1. Effects of cyanide produced by human isolates of *Pseudomonas aeruginosa* on *Drosophila melanogaster*. In panel A, 3 human isolates of *P. aeruginosa*, referred to as strains A, B, and C, and *Escherichia coli* strain DH-5α were grown to confluency on Luria-Bertani agar in the bottom of closed glass jars (9 cm in diameter). The jars were opened for 10 min to allow equilibration with ambient air and then closed; cyanide gas emitted from the cultures during a 1-h period was collected in NaOH and measured as described in Methods. Values for strains B and C were significantly higher than those for strain A or for the agar-only plate (*P* < 0.01), and values for strain C were significantly higher than those for strain B (*P* < 0.05). In panel B, 10 fruit flies (*D. melanogaster*) were placed in a small glass cylinder stoppered at both ends with glass wool. The cylinder was suspended above bacteria in the glass jars described above, the jars were placed in a 37°C room, and the flies were observed for activity every 10 min for 100 min. Some of the flies had previously been grown on food containing 100 μmol/L cobinamide (Cbi) (black symbols); Cbi had no effect on flies exposed to *P. aeruginosa* strain A. At 100 min, results were significantly different in flies exposed to strain B or C than in those exposed to strain A, and Cbi-treated flies differed from untreated flies for strains B and C (*P* < 0.05, for all). In panel C, the cylinder containing the flies shown in panel B was removed from the glass jars and placed in room-temperature ambient air. The no. of active flies was scored every 10 min for 150 min for flies exposed to *P. aeruginosa* strain A, B, or C. Even after 150 min, flies exposed to strain C showed significantly less activity than flies exposed to strain A or B; flies exposed to strain B were significantly less active than strain A–exposed flies, even up to 100 min (*P* < 0.01). In panel D, 10 flies were exposed to bacteria or agar only and were allowed to recover from the exposure, as described for panels B and C, respectively. After a 100-min recovery period, heart rates were measured, as described in Methods. Flies exposed to strain B or C had significantly lower heart rates than flies exposed to agar only or strain A (*P* < 0.05), but there was no difference in heart rates between flies exposed to strain B and those exposed to strain C. In panel E, 5 flies exposed to bacteria or agar for 100 min, as described for panel B, were decapitated and extracted in NaOH, and cyanide was measured in the extracts. Black bars represent flies that had been grown on Cbi. Flies exposed to strain B or C had significantly higher cyanide levels than those exposed to agar only or to strain A, and growing flies on Cbi significantly reduced cyanide levels in flies exposed to strain B or C (*P* < 0.05). All experiments were performed at least 3 times, and data are means ± SDs.
ing *P. aeruginosa* strain C into flies reduced their ATP concentration by 55%, whereas injecting strain A or *E. coli* had no significant effect; injecting flies with strain B had an intermediate effect on the ATP concentration (figure 3B). The correlation between cyanide production by the 3 *Pseudomonas* strains and their effects on ATP suggests that cyanide caused the decrease in ATP concentration. Moreover, the ATP reduction observed in strain B– and strain C–infected flies was prevented by coinjecting cobinamidie, providing further evidence that the effect on ATP was likely mediated via cyanide (figure 3B). For comparison, we exposed flies to cyanide at 22 ppm [14] and found that this lethal dose of cyanide reduced ATP concentrations, with partial restoration by cobinamide (figure 3B).

![Figure 2](https://academic.oup.com/jid/article-abstract/197/3/457/2908685)

**Figure 2.** Survival of *Drosophila melanogaster* injected with *Pseudomonas aeruginosa*. Flies were injected in the thorax with ~5 × 10⁸ *P. aeruginosa* in 1 μL of broth; a 33-gauge needle was used on a 2.5-μL syringe. Fly survival was monitored for the indicated times. For each condition, at least 10 flies were injected per experiment; the data are means ± SDs of 3 independent experiments. In panel A, flies were injected with *P. aeruginosa* strain A, B, or C. Black symbols show flies for which 500 μmol/L cobinamide (Cbi) was added to the bacterial broth. Fly survival at 48 h was significantly less for flies injected with strain B or C than for flies injected with strain A (P < .01), and Cbi significantly increased survival in flies injected with strain B or C (P < .05) but had no effect on survival of flies injected with strain A. In panel B, all of the flies were injected with *P. aeruginosa* strain C, alone or with the addition of 500 μmol/L Cbi, 500 μmol/L hydroxocobalamin (cobalamin), or 100 μmol/L sodium thiosulfate. All 3 cyanide-neutralizing agents significantly increased fly survival at 48 h (P < .05).

![Figure 3](https://academic.oup.com/jid/article-abstract/197/3/457/2908685)

**Figure 3.** Effect of *Pseudomonas aeruginosa* on nitrite, nitrate, and adenosine triphosphate (ATP) concentrations in *Drosophila melanogaster*. *D. melanogaster* were injected with either bacterial broth (bars at far left) or ~5 × 10⁸ *P. aeruginosa* strain A, B, or C; 3 h later, they were decapitated and extracted using a commercial lysis buffer (A) or 0.4N perchloric acid (B). Nitrite and nitrate (A) and ATP (B) were measured in the extracts, as described in Methods. Black bars show flies co-injected with 500 μmol/L cobinamide (Cbi). Panel B includes flies injected with ~5 × 10⁸ *E. coli* and un.injected flies exposed to 22-ppm cyanide gas for 1 min. For both panels A and B, 5 flies were injected in each experiment. There was no significant difference in nitrite and nitrate levels among flies injected with the 3 *Pseudomonas* strains (P > .2), but Cbi significantly lowered nitrite and nitrate levels in flies injected with any of the strains (P < .05). The ATP content was lowered significantly in flies injected with *Pseudomonas* strain C (P < .05), but the reduction in ATP content in flies injected with *Pseudomonas* strain B did not reach statistical significance (P = .1). Cbi significantly increased ATP in the flies injected with *Pseudomonas* strain C (P < .05). Data are means ± SDs for at least 3 independent experiments.
Lower Virulence to Drosophila Flies of P. aeruginosa Deficient in Cyanide Production

To study further the role played by P. aeruginosa cyanide production in Drosophila flies, we injected flies with P. aeruginosa strain PAO1 in which the hcnC gene had been mutated (strain MP507 [11]). In preliminary experiments, we showed that the parental wild-type strain produced significant amounts of cyanide, similar to strain B, whereas the hcnC mutant produced no measurable cyanide. The parental wild-type strain killed flies within 24 h, whereas the hcnC mutant killed less than half of the flies during the same time interval (figure 4). To confirm that the difference in virulence between the parental PAO1 strain and the hcnC mutant was due to cyanide production, we injected flies with the hcnC mutant that had the hcnABC gene cluster reinserted [11]. Two different knock-in strains were used (strain MP507 transformed with pLG2 and pLG4 [11]), and both killed the flies with kinetics almost identical to those of the wild-type parental strain (figure 4; cyanide production by the 2 knock-in strains was similar to that of the parental strain). These data provide strong evidence that cyanide contributes to P. aeruginosa virulence in Drosophila flies.

Resistance of Flies Overexpressing Bovine Rhodanese to P. aeruginosa Toxicity

A major advantage of studying D. melanogaster is the ease of generating genetic mutants. Because the enzyme rhodanese detoxifies cyanide, we generated flies overexpressing bovine rhodanese to determine whether they exhibited resistance to P. aeruginosa toxicity. We generated 8 different transgenic fly lines, each overexpressing rhodanese to some degree, and chose 2 of the higher rhodanese-expressing lines (figure 5A).

We demonstrated previously that exposing flies for 1 min to 22-ppm cyanide gas causes the flies to fall motionless [14]. Wild-type untreated flies never recover activity after removal from the cyanide, whereas flies grown on cobinamide regain full activity within ~30 min after removal from the cyanide [14]. We found that the 2 transgenic fly lines overexpressing bovine rhodanese recovered from cyanide exposure with kinetics similar to those in cobinamide-fed wild-type flies, whereas untreated wild-type flies remained motionless and died (figure 5B). Thus, the rhodanese-overexpressing flies exhibited cyanide resistance.

To determine whether the rhodanese-overexpressing flies were less vulnerable to cyanide-producing Pseudomonas strains than wild-type flies, we injected the flies with bacteria and monitored survival. Wild-type flies injected with P. aeruginosa strains A, B, and C died during the same time periods shown in figure 2A, with all of the flies injected with strain C being dead within 30 h after injection (figure 5C; the flies used in these experiments were Canton-S, but similar results were found with the w1118 parental strain of the rhodanese-overexpressing flies). Wild-type and rhodanese-overexpressing flies were equally susceptible to strain A, but the rhodanese-overexpressing flies survived significantly longer than wild-type flies when injected with strain C; the rhodanese-overexpressing flies showed increased resistance to strain B, but the difference did not reach statistical significance (figure 5C; data are for rhodanese transgenic line 1, but similar results were obtained with line 2).

DISCUSSION

P. aeruginosa infection is associated with high rates of morbidity and mortality, and, in a recent review of sepsis, P. aeruginosa was the only organism that independently portended a poor prognosis [27]. Similarly, in a review of nosocomial bacteremia, P. aeruginosa was associated with the highest mortality rates among bacteria, equal to that of Candida infections [28]. In patients with cystic fibrosis, P. aeruginosa develops a mucoid phenotype over time by secreting the polysaccharide alginate [29, 30]. Acquisition of the mucoid phenotype signals significant exacerbation in the lung disease of patients with cystic fibrosis, and mucoid P. aeruginosa strains produce more cyanide than nonmucoid strains; these data suggest that cyanide may contribute to the deterioration in lung function in P. aeruginosa–infected patients with cystic fibrosis [4].

Gallagher and Manoil showed elegantly that P. aeruginosa strain PAO1 kills C. elegans by cyanide poisoning [11]. C. elegans lacks a heart, and its nervous system contains only 300 neurons. We chose D. melanogaster as a model system for studying cyanide toxicity because the organism has a heart/circulatory system and brain/nervous system [22]. Moreover, we demonstrated previously that D. melanogaster is a good model for...
studying the effects of cyanide [14]. In experiments in which \textit{P. aeruginosa} was injected into flies, resembling a clinical model of sepsis [20, 31], we found good evidence that cyanide produced by the bacterium was contributing to fly mortality. These experiments included both new isolates of \textit{P. aeruginosa} from humans as well as the established PAO1 strain. The latter provided the opportunity to study bacteria with a mutation in the \textit{hcnC} gene; these bacteria were much less toxic to \textit{Drosophila} flies than the parental wild-type strain and 2 different knock-in strains. Cyanide production was clearly not the only mechanism of virulence in the flies, however, because strain A, which produced little or no cyanide, killed flies, albeit with slower kinetics than strain B or C. Similarly, the \textit{hcnC} PAO1 mutant, which produced no detectable cyanide, exhibited some toxicity to the flies.

Because we do not have clinical histories for the patients from whom strains A, B, and C were isolated, we cannot compare the virulence in our experimental fly model to that in humans. However, as mentioned earlier, the virulence of various \textit{P. aeruginosa} mutants in insects correlates well with that in mice [15]. If this correlation extends to humans, then perhaps high-cyanide-producing strains of \textit{P. aeruginosa} are more toxic to humans than low-cyanide-producing strains, as suggested by a study of burn patients [13].

If cyanide contributes to the pathogenicity of \textit{P. aeruginosa}, then a rational treatment of infections caused by this organism could include a cyanide-detoxifying agent. Consistent with this idea, we found that cobinamide improved survival of flies injected with cyanide-producing strains of \textit{P. aeruginosa}. Cobinamide has an extremely high affinity for cyanide (overall binding
affinity of $\sim 10^{12}$ [mol/L]−1) [32], and we showed previously that it is a very effective cyanide-detoxifying agent, more effective than hydroxocobalamin; the latter was recently approved by the Food and Drug Administration for treating cyanide-poisoned patients [14, 33]. Cobinamide rescued P. aeruginosa-injected flies more effectively than sodium thiosulfate, another clinically approved cyanide-detoxifying agent [34]. Cobinamide might be particularly effective in cases of sepsis caused by cyanogenic P. aeruginosa, because it could neutralize cyanide and pathologically elevated NO concentrations; we demonstrated previously that cobinamide was beneficial in E. coli and S. aureus sepsis in flies [20]. At the concentrations used in the present work, cobinamide did not appear to function by binding NO, because nitrite and nitrate concentrations were similar in the low-, medium-, and high-cyanide-producing P. aeruginosa strains.

Transgenic flies overexpressing bovine rhodanese exhibited resistance to cyanide and to cyanide-producing strains of P. aeruginosa. The equal susceptibility of the transgenic and wild-type flies to the non–cyanide-producing P. aeruginosa strain suggests that the transgenic flies did not have a general resistance to Pseudomonas infection. Thus, these flies provided a good model to demonstrate that cyanide clearly contributed to the virulence of the high-cyanide-producing strains of P. aeruginosa. Work is in progress to determine whether cyanide contributes to pathogenicity in a mammalian model of P. aeruginosa infection.

Acknowledgments

We thank Dr. Larry Gallagher for generously providing the PAO1 wild-type, hcnC mutant, and hcnABC knock-in strains.

References