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Quorum-Quenching Acylase Reduces the Virulence of *Pseudomonas aeruginosa* in a *Caenorhabditis elegans* Infection Model[▽]

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The *Pseudomonas aeruginosa* PAO1 gene *pvdQ* encodes an acyl-homoserine lactone (AHL) acylase capable of degrading *N*-(3-oxododecanoyl)-L-homoserine lactone by cleaving the AHL amide. PvdQ has been proven to function as a quorum quencher in vitro in a number of phenotypic assays. To address the question of whether PvdQ also shows quorum-quenching properties in vivo, an infection model based on the nematode *Caenorhabditis elegans* was explored. In a fast-acting paralysis assay, strain PAO1(pME*pvdQ*), which overproduces PvdQ, was shown to be less virulent than the wild-type strain. More than 75% of the nematodes exposed to PAO1(pME*pvdQ*) survived and continued to grow when using this strain as a food source. Interestingly, in a slow-killing assay monitoring the survival of the nematodes throughout a 4-day course, strain PAO1-Δ*pvdQ* was shown to be more virulent than the wild-type strain, confirming the role of PvdQ as a virulence-reducing agent. It was observed that larval stage 1 (L1) to L3-stage larvae benefit much more from protection by PvdQ than L4 worms. Finally, purified PvdQ protein was added to *C. elegans* worms infected with wild-type PAO1, and this resulted in reduced pathogenicity and increased the life span of the nematodes. From our observations we can conclude that PvdQ might be a strong candidate for antibacterial therapy against *Pseudomonas* infections.

Pseudomonas aeruginosa is an opportunistic gram-negative pathogen of vertebrates and a primary pathogen of insects (17). It mainly infects individuals who are immunocompromised, such as human immunodeficiency virus-infected patients, as well as those who have cystic fibrosis. In addition, those having disruptions in normal barriers caused by severe burns or indwelling medical devices are at risk. Hospital-acquired *P. aeruginosa* pneumonias and septicemias are frequently lethal (2, 3). To facilitate the establishment of infection, *P. aeruginosa* produces an impressive array of both cell-associated and extracellular virulence factors, such as proteases and phospholipases, and also small molecules, including rhamnolipid, phenazines, and cyanide (17). Expression of many of the extracellular factors is cell density controlled, does not occur until the late logarithmic phase of growth, and is mediated through specific quorum-sensing signal molecules (23). Two of these molecules, *N*-butanoyl-L-homoserine lactone (C4-HSL) and *N*-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-C12-HSL), have been studied in great detail. In our laboratory, we previously demonstrated that PA2385(*pvdQ*) from *P. aeruginosa* PAO1 encodes an acyl-homoserine lactone (AHL) acylase. Analysis of the gene product showed that the posttranslational processing of the acylase as well as the hydrolysis reaction type are similar to those of the beta-lactam acylases, strongly suggesting that the PvdQ protein is a member of the N-terminal nucleophile hydrolase superfamily. The main AHL signaling molecule of *P. aeruginosa* PAO1, 3-oxo-C12-HSL, is degraded by PvdQ (16). Addition of the purified protein to PAO1 cultures completely inhibited accumulation of 3-oxo-C12-HSL and production of the signal molecule 2-heptyl-3-hydroxy-4(1*H*)-quinolone and reduced production of the virulence factors elastase and pyocyanin. Similar results were obtained when *pvdQ* was overexpressed in *P. aeruginosa* (16). These results demonstrate that this protein has in situ quorum-quenching activity. This AHL acylase may enable *P. aeruginosa* PAO1 to modulate its own quorum-sensing-dependent pathogenic potential and, moreover, offers possibilities for novel antipseudomonal therapies.

To test our hypothesis that PvdQ can exert its beneficial functions also in vivo, we chose to study its effect on the infection of the nematode *Caenorhabditis elegans*. This model has been used before in multiple pathogenicity studies of *Cryptococcus neoformans* (13) and gram-positive (6) and gram-negative (9, 10, 11) bacteria. Infection with *P. aeruginosa* strain PA14 was found to result in fast (hours) or slow (days) killing, depending on the growth medium used (19, 20). When Darby and colleagues (2) used the system to study *P. aeruginosa* PAO1, a lethal paralysis of the worms was observed, indicating another mechanism by which *P. aeruginosa* can kill *C. elegans.* It was shown that quorum-sensing-dependent hydrogen cyanide production on rich medium by *P. aeruginosa* PAO1 is the causative agent for the fast paralysis (5). Under the same conditions, an attenuation of paralysis by an AHL acylase from *Ralstonia* sp. strain XJ12B upon expression in *P. aeruginosa*

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