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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)-i-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(pMEpvdQ), which overproduces PvdQ, was
shown to be less virulent than the wild-type strain. More than 75% of the nematodes exposed to
PAO1(pMEpvdQ) 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-ΔpvdQ
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 immunocompro-
mised, such as human immunodeficiency virus-infected pa-
ents, 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-ac-
quired P. aeruginosa pneumonias and septicemias are fre-
cently lethal (2, 3). To facilitate the establishment of infec-
tion, P. aeruginosa produces an impressive array of both
cell-associated and extracellular virulence factors, such as
proteases and phospholipases, and also small molecules, in-cluding
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-butyl-3-(i-homoserine lact-
one (C4-HSL) and N-(3-oxododecanoyl)-i-homoserine lact-
one (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 hy-
drolysis reaction type are similar to those of the beta-lactam
acylases, strongly suggesting that the PvdQ protein is a mem-
er 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(1H)-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-depen-
dent 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 Cryp-
tococcus 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 cyan-
ide 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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