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PA0305 of *Pseudomonas aeruginosa* is a quorum quenching acylhomoserine lactone acylase belonging to the Ntn hydrolase superfamily

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The *Pseudomonas aeruginosa* PAO1 genome has at least two genes, *pvdQ* and *quiP,* encoding acylhomoserine lactone (AHL) acylases. Two additional genes, *pa1893* and *pa0305*, have been predicted to encode penicillin acylase proteins, but have not been characterized. Initial studies on a *pa0305* transposon insertion mutant suggested that the gene is not related to the AHL growth phenotype of *P. aeruginosa*. The close similarity (67 %) of *pa0305* to HacB, an AHL acylase of *Pseudomonas syringae*, prompted us to investigate whether the PA0305 protein might also function as an AHL acylase. The *pa0305* gene has been cloned and the protein (PA0305) has been overproduced, purified and subjected to functional characterization. Analysis of the purified protein showed that, like β-lactam acylases, PA0305 undergoes post-translational processing resulting in α- and β-subunits, with the catalytic serine as the first amino acid of the β-subunit, strongly suggesting that PA0305 is a member of the N-terminal nucleophile hydrolase superfamily. Using a biosensor assay, PA0305his was shown to degrade AHLs with acyl side chains ranging in length from 6 to 14 carbons. Kinetics studies using *N*-octanoyl-L-homoserine lactone (C_8-HSL) and *N*-(3-oxo-dodecanoyl)-L-homoserine lactone (3-oxo-C_{12}-HSL) as substrates showed that the enzyme has a robust activity towards these two AHLs, with apparent K_{cat}/K_m values of 0.14×10^4 M^{-1} s^{-1} towards C_8-HSL and 7.8×10^4 M^{-1} s^{-1} towards 3-oxo-C_{12}-HSL. Overexpression of the *pa0305* gene in *P. aeruginosa* showed significant reductions in both accumulation of 3-oxo-C_{12}-HSL and expression of virulence factors. A mutant *P. aeruginosa* strain with a deleted *pa0305* gene showed a slightly increased capacity to kill *Caenorhabditis elegans* compared with the *P. aeruginosa* PAO1 wild-type strain and the PAO1 strain carrying a plasmid overexpressing *pa0305*. The harmful effects of the Δ*pa0305* strain on the animals were most visible at 5 days post-exposure and the mortality rate of the animals fed on the Δ*pa0305* strain was faster than for the animals fed on either the wild-type strain or the strain overexpressing *pa0305*. In conclusion, the *pa0305* gene encodes an efficient acylase with activity towards long-chain homoserine lactones, including 3-oxo-C_{12}-HSL, the natural quorum sensing signal molecule in *P. aeruginosa,* and we propose to name this acylase HacB.

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INTRODUCTION

Pseudomonas aeruginosa is an opportunistic pathogen, often detected in immune-compromised patients and hospital-acquired infections. A high percentage of cystic fibrosis patients acquire chronic *P. aeruginosa* infections leading to high mortality rates within this group (Lyczak *et al.*, 2000; Tatterson *et al.*, 2001). *P. aeruginosa* employs a complex network of quorum sensing (QS) systems necessary to control expression of density-dependent genes, including genes encoding virulence factors. Induction of these genes depends on production, secretion and detection of the 3-oxo-C_{12}-HSL and the C_4-HSL signal molecules [full abbreviations for all the acylhomoserine lactones (AHLs) used in this study are given in Table 1]. The high

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Abbreviations: AHL, acylhomoserine lactone; BrMMC, 4-bromomethoxy-7-methyl coumarin; HSL, homoserine lactone; QS, quorum sensing; RLU, relative light units.

A supplementary figure, illustrating detection of AHLs with TLC, is available with the online version of this paper.

