



# Commentary: The *icmF3* Locus is Involved in Multiple Adaptation- and Virulence-related Characteristics in *Pseudomonas aeruginosa* PAO1

Le Tang, Xiaoye Liang, Richard Moore and Tao G. Dong\*

Ecosystem and Public Health, University of Calgary, Calgary, AB, Canada

**Keywords:** *Pseudomonas aeruginosa*, PAO1, antimicrobial activity, T6SS, IcmF, strain divergence

## A commentary on

The *icmF3* locus is involved in multiple adaptation- and virulence-related characteristics in *Pseudomonas aeruginosa* PAO1

by Lin, J., Cheng, J., Chen, K., Guo, C., Zhang, W., Yang, X., et al. (2015). *Front. Cell. Infect. Microbiol.* 5:70. doi: 10.3389/fcimb.2015.00070

*Pseudomonas aeruginosa* is an important pathogen commonly isolated from patients with burns, wounds and cystic fibrosis (Lyczak et al., 2000; Gellatly and Hancock, 2013). The *P. aeruginosa* strain PAO1 was originally reported as a wound isolate from a patient in Australia in 1955 (Holloway, 1955), and has since been studied in many laboratories as a reference strain (Stover et al., 2000). However, a number of genetic variants of PAO1 in different laboratories have been reported including a large 2.2 Mb inversion and a number of single nucleotide variants and insertion-deletion mutations (Stover et al., 2000; Heurlier et al., 2005; Klockgether et al., 2010).

The type 6 secretion system (T6SS) functions as a molecular weapon that delivers toxic effectors to prokaryotic and eukaryotic target cells (Ho et al., 2014). The T6SS was first functionally characterized in *Vibrio cholerae* and *P. aeruginosa* PAO1 by the Mekalanos group in 2006 (Mougous et al., 2006; Pukatzki et al., 2006), and thereafter the PAO1 strain has been used as an important model to study the T6SS functions (Ho et al., 2014; Russell et al., 2014). PAO1 possesses three distinct T6SS clusters (H1, H2, and H3) of which the H1-T6SS delivers six known antimicrobial substrates (Hood et al., 2010; Whitney et al., 2014). The H2- and the H3-T6SS are implicated in both antimicrobial and anti-eukaryotic activities and can secrete PldA and PldB phospholipases, respectively (Lesic et al., 2009; Sana et al., 2012; Russell et al., 2013; Jiang et al., 2014).

The T6SS main structure consists of an outer sheath, an inner tube, and a membrane-bound anchor complex (Basler, 2015). Contraction of the outer sheath ejects the inner tube and its associated effector proteins to the extracellular environment (Ho et al., 2014; Basler, 2015). IcmF, a key T6SS protein, carries an ATPase domain (Ma et al., 2012) and interacts with TssL and TssJ to form a membrane-spanning complex with a hollow space that allows the inner tube and effectors to travel through (Basler, 2015; Durand et al., 2015). The recently published paper by Lin et al. (Lin et al., 2015) reported multiple interesting yet moderate phenotypes associated with the IcmF3 of the H3-T6SS, including iron acquisition, bacterial killing, motility, antibiotic resistance, and virulence. However, the molecular mechanism of IcmF3 involvement in such diverse cellular processes is not clear. Because IcmF is required for T6SS assembly, those reported phenotypes suggest a broad versatile role of the H3-T6SS in PAO1

## OPEN ACCESS

### Edited by:

Matthew S. Francis,  
Umeå University, Sweden

### Reviewed by:

Michael L. Vasil,  
University of Colorado School of  
Medicine, USA  
Annabelle Merieau,  
University of Rouen, France

### \*Correspondence:

Tao G. Dong  
tdong@ucalgary.ca

**Received:** 20 October 2015

**Accepted:** 03 November 2015

**Published:** 18 November 2015

### Citation:

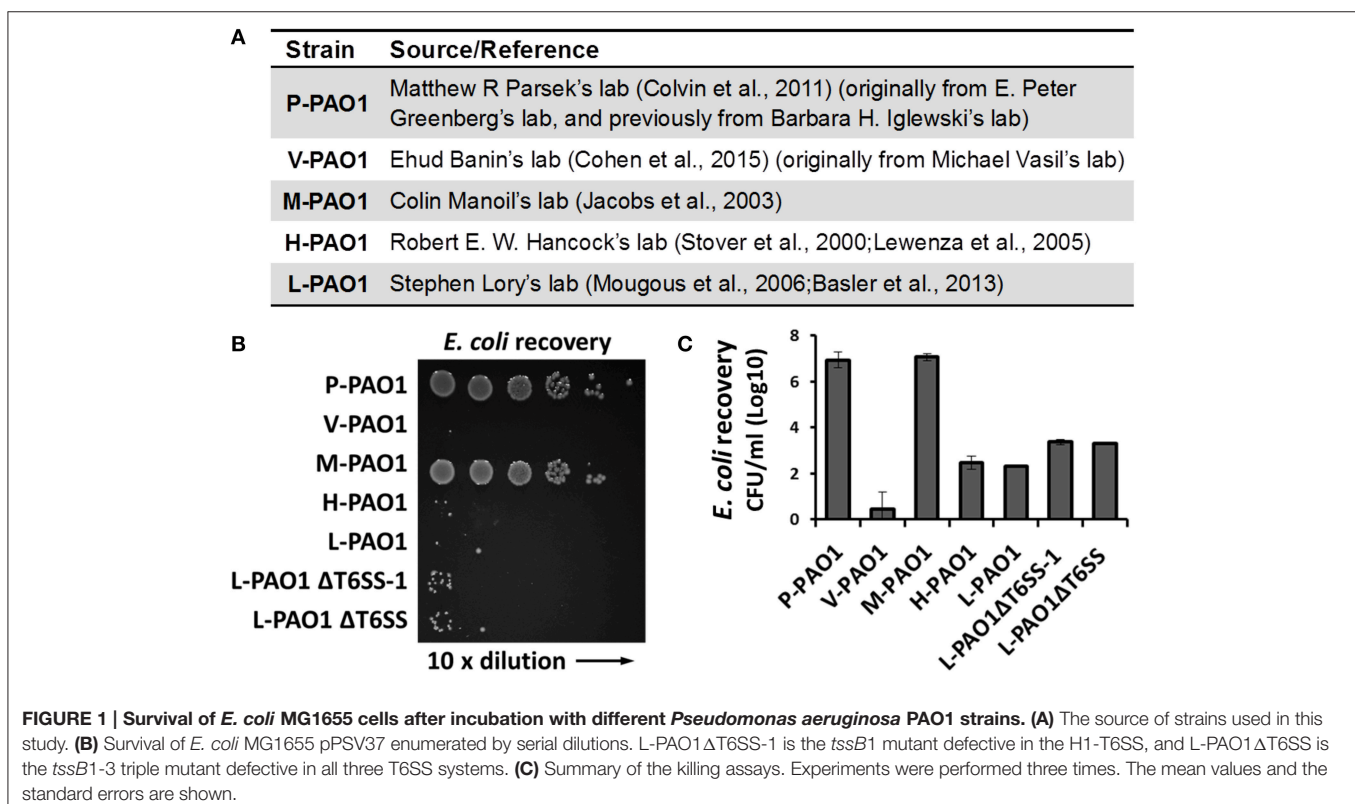
Tang L, Liang X, Moore R and  
Dong TG (2015) Commentary: The  
*icmF3* Locus is Involved in Multiple  
Adaptation- and Virulence-related  
Characteristics in *Pseudomonas*  
*aeruginosa* PAO1.  
*Front. Cell. Infect. Microbiol.* 5:83.  
doi: 10.3389/fcimb.2015.00083

(Lin et al., 2015). Interestingly, the T6SS in *Yersinia pseudotuberculosis* is involved in  $Zn^{2+}$  transportation (Wang et al., 2015). An alternative but not mutually exclusive explanation is that IcmF3 itself regulates other cellular processes in addition to its primary role as a key T6SS component.

Lin et al. tested *Escherichia coli* survival after co-incubation with PAO1 or the *icmF3* mutant for 36 h and reported that the CFU of *E. coli* remained at a high level ( $10^8$  CFU/ml, see Table S3 in Lin et al., 2015). We found this surprising because the PAO1 strain in our lab (L-PAO1), obtained from J. Mekalanos (Mougous et al., 2006; Basler et al., 2013) and originally from S. Lory, can efficiently kill *E. coli* cells after 24 h co-incubation. Considering the reported genome divergence of PAO1 in different laboratories (Klockgether et al., 2010), we hypothesized that different PAO1 sublines may have variable killing abilities against *E. coli*. To test this hypothesis, we carried out *E. coli* killing assays using several PAO1 strains from different sources (Figure 1A), of which the L-PAO1 has been used extensively in T6SS research (Mougous et al., 2006; Hood et al., 2010; Basler et al., 2013; Ho et al., 2013). The H-PAO1 and the M-PAO1 are isolates from R. Hancock and C. Manoil, respectively, and are the host strains for two defined PAO1 transposon mutant libraries (Jacobs et al., 2003; Lewenza et al., 2005). The P-PAO1 strain and the V-PAO1 strain are from M. Parsek (Colvin et al., 2011) and E. Banin (Cohen et al., 2015), respectively. We followed the reported protocol by Lin et al. (2015) with minor modifications, primarily that

the killing was done on LB medium directly instead of a filter membrane. Overnight cultures of PAO1 and *E. coli* MG1655 carrying a pPSV37 plasmid vector (gentamycin resistance) (Lee et al., 2010) were washed with fresh LB and then mixed at a 10 to 1 ratio, followed by co-incubation on a LB agar plate at 37°C for 36 h. Survival of *E. coli* was enumerated by serial dilutions on LB-gentamycin plates. Our results demonstrate that L-PAO1, V-PAO1, and H-PAO1 eliminated *E. coli* MG1655 after co-incubation, whereas P-PAO1 and M-PAO1 had little killing activity against *E. coli* (Figures 1B,C). To test if the T6SS is required for the observed killing, we constructed the *tssB1* deletion mutant ( $\Delta H1$ -T6SS) and the *tssB1*-3 triple deletion mutant ( $\Delta T6SS$ ) in the L-PAO1 strain. Our results show that both mutants killed *E. coli* efficiently, suggesting L-PAO1 possesses other antimicrobial mechanisms independent of the T6SS clusters.

The genome divergence of different PAO1 strains is known to cause phenotypic variations in virulence (Preston et al., 1995; Klockgether et al., 2010). Here we show different PAO1 strains also differ in their capability of killing neighboring cells. In complex multispecies environments such as the cystic fibrosis patient's lung, it is conceivable that competition between different species may select for PAO1 mutants with enhanced killing abilities. However, how PAO1 strains during lab passage diverge to gain/lose antimicrobial properties is not intuitively apparent. Nonetheless, because PAO1 is widely used as a model strain, researchers should be aware of the strain variations and should provide detailed description of the strain source



to allow the *P. aeruginosa* community to better interpret the results.

## AUTHOR CONTRIBUTIONS

LT, XL, RM performed the experiments. TD conceived the study and designed the experiment. LT and TD wrote the paper.

## FUNDING STATEMENT

This work was supported by a start-up grant of the University of Calgary and a Canadian Institutes of Health Research (CIHR)

operating grant to TD. TD is also supported by a Government of Canada Research Chair award, a Canadian Foundation for Innovation grant (CFI-JELF), and an Alberta Innovation and Advanced Education grant.

## ACKNOWLEDGMENTS

We are grateful to John Mekalanos for providing L-PAO1, Shawn Lewenza for H-PAO1 and Joe J. Harrison for P-PAO1, M-PAO1, and V-PAO1 strains. We thank the Advancing Canadian Wastewater Assets (ACWA) for providing infrastructure support.

## REFERENCES

- Basler, M. (2015). Type VI secretion system: secretion by a contractile nanomachine. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 370:20150021. doi: 10.1098/rstb.2015.0021
- Basler, M., Ho, B. T., and Mekalanos, J. J. (2013). Tit-for-tat: type VI secretion system counterattack during bacterial cell-cell interactions. *Cell* 152, 884–894. doi: 10.1016/j.cell.2013.01.042
- Cohen, D., Mechold, U., Nevenzal, H., Yarmiyhu, Y., Randall, T. E., Bay, D. C., et al. (2015). Oligoribonuclease is a central feature of cyclic diguanylate signaling in *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. U.S.A.* 112, 11359–11364. doi: 10.1073/pnas.1421450112
- Colvin, K. M., Gordon, V. D., Murakami, K., Borlee, B. R., Wozniak, D. J., Wong, G. C. L., et al. (2011). The pel polysaccharide can serve a structural and protective role in the biofilm matrix of *Pseudomonas aeruginosa*. *PLoS Pathog.* 7:e1001264. doi: 10.1371/journal.ppat.1001264
- Durand, E., Nguyen, V. S., Zoued, A., Logger, L., Péhau-Arnaudet, G., Aschtgen, M.-S., et al. (2015). Biogenesis and structure of a type VI secretion membrane core complex. *Nature* 523, 555–560. doi: 10.1038/nature14667
- Gellatly, S. L., and Hancock, R. E. W. (2013). *Pseudomonas aeruginosa*: new insights into pathogenesis and host defenses. *Pathog. Dis.* 67, 159–173. doi: 10.1111/2049-632X.12033
- Heurlier, K., Déneraud, V., Haenni, M., Guy, L., Krishnapillai, V., and Haas, D. (2005). Quorum-sensing-negative (lasR) mutants of *Pseudomonas aeruginosa* avoid cell lysis and death. *J. Bacteriol.* 187, 4875–4883. doi: 10.1128/JB.187.14.4875-4883.2005
- Ho, B. T., Basler, M., and Mekalanos, J. J. (2013). Type 6 secretion system-mediated immunity to type 4 secretion system-mediated gene transfer. *Science* 342, 250–253. doi: 10.1126/science.1243745
- Ho, B. T., Dong, T. G., and Mekalanos, J. J. (2014). A view to a kill: the bacterial type VI secretion system. *Cell Host Microbe* 15, 9–21. doi: 10.1016/j.chom.2013.11.008
- Holloway, B. W. (1955). Genetic recombination in *Pseudomonas aeruginosa*. *J. Gen. Microbiol.* 13, 572–581. doi: 10.1099/00221287-13-3-572
- Hood, R. D., Singh, P., Hsu, F., Güvener, T., Carl, M. A., Trinidad, R. R. S., et al. (2010). A type VI secretion system of *Pseudomonas aeruginosa* targets a toxin to bacteria. *Cell Host Microbe* 7, 25–37. doi: 10.1016/j.chom.2009.12.007
- Jacobs, M. A., Alwood, A., Thaipisuttikul, I., Spencer, D., Haugen, E., Ernst, S., et al. (2003). Comprehensive transposon mutant library of *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. U.S.A.* 100, 14339–14344. doi: 10.1073/pnas.2036282100
- Jiang, F., Waterfield, N. R., Yang, J., Yang, G., and Jin, Q. (2014). A *Pseudomonas aeruginosa* type VI secretion phospholipase D effector targets both prokaryotic and eukaryotic cells. *Cell Host Microbe* 15, 600–610. doi: 10.1016/j.chom.2014.04.010
- Klockgether, J., Munder, A., Neugebauer, J., Davenport, C. F., Stanke, F., Larbig, K. D., et al. (2010). Genome diversity of *Pseudomonas aeruginosa* PAO1 laboratory strains. *J. Bacteriol.* 192, 1113–1121. doi: 10.1128/JB.01515-09
- Lee, P.-C., Stopford, C. M., Svenson, A. G., and Rietsch, A. (2010). Control of effector export by the *Pseudomonas aeruginosa* type III secretion proteins PcrG and PcrV. *Mol. Microbiol.* 75, 924–941. doi: 10.1111/j.1365-2958.2009.07027.x
- Lesic, B., Starkey, M., He, J., Hazan, R., and Rahme, L. G. (2009). Quorum sensing differentially regulates *Pseudomonas aeruginosa* type VI secretion locus I and homologous loci II and III, which are required for pathogenesis. *Microbiol. Read. Engl.* 155, 2845–2855. doi: 10.1099/mic.0.029082-0
- Lewenza, S., Falsafi, R. K., Winsor, G., Gooderham, W. J., McPhee, J. B., Brinkman, F. S. L., et al. (2005). Construction of a mini-Tn5-luxCDABE mutant library in *Pseudomonas aeruginosa* PAO1: a tool for identifying differentially regulated genes. *Genome Res.* 15, 583–589. doi: 10.1101/gr.3513905
- Lin, J., Cheng, J., Chen, K., Guo, C., Zhang, W., Yang, X., et al. (2015). The *icmF3* locus is involved in multiple adaptation- and virulence-related characteristics in *Pseudomonas aeruginosa* PAO1. *Front. Cell. Infect. Microbiol.* 5:70. doi: 10.3389/fcimb.2015.00070
- Lyczak, J. B., Cannon, C. L., and Pier, G. B. (2000). Establishment of *Pseudomonas aeruginosa* infection: lessons from a versatile opportunist. *Microbes Infect. Inst. Pasteur* 2, 1051–1060. doi: 10.1016/S1286-4579(00)01259-4
- Ma, L.-S., Narberhaus, F., and Lai, E.-M. (2012). IcmF family protein TssM exhibits ATPase activity and energizes type VI secretion. *J. Biol. Chem.* 287, 15610–15621. doi: 10.1074/jbc.M111.301630
- Mougous, J. D., Cuff, M. E., Raunser, S., Shen, A., Zhou, M., Gifford, C. A., et al. (2006). A virulence locus of *Pseudomonas aeruginosa* encodes a protein secretion apparatus. *Science* 312, 1526–1530. doi: 10.1126/science.1128393
- Preston, M. J., Fleiszig, S. M., Zaidi, T. S., Goldberg, J. B., Shortridge, V. D., Vasil, M. L., et al. (1995). Rapid and sensitive method for evaluating *Pseudomonas aeruginosa* virulence factors during corneal infections in mice. *Infect. Immun.* 63, 3497–3501.
- Pukatzki, S., Ma, A. T., Sturtevant, D., Krastins, B., Sarracino, D., Nelson, W. C., et al. (2006). Identification of a conserved bacterial protein secretion system in *Vibrio cholerae* using the Dictyostelium host model system. *Proc. Natl. Acad. Sci. U.S.A.* 103, 1528–1533. doi: 10.1073/pnas.0510322103
- Russell, A. B., LeRoux, M., Hathazi, K., Agnello, D. M., Ishikawa, T., Wiggins, P. A., et al. (2013). Diverse type VI secretion phospholipases are functionally plastic antibacterial effectors. *Nature* 496, 508–512. doi: 10.1038/nature12074
- Russell, A. B., Peterson, S. B., and Mougous, J. D. (2014). Type VI secretion system effectors: poisons with a purpose. *Nat. Rev. Microbiol.* 12, 137–148. doi: 10.1038/nrmicro3185
- Sana, T. G., Hachani, A., Bucior, I., Soscia, C., Garvis, S., Termine, E., et al. (2012). The second type VI secretion system of *Pseudomonas aeruginosa* strain PAO1 is regulated by quorum sensing and Fur and modulates internalization in epithelial cells. *J. Biol. Chem.* 287, 27095–27105. doi: 10.1074/jbc.M112.376368

- Stover, C. K., Pham, X. Q., Erwin, A. L., Mizoguchi, S. D., Warren, P., Hickey, M. J., et al. (2000). Complete genome sequence of *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen. *Nature* 406, 959–964. doi: 10.1038/35023079
- Wang, T., Si, M., Song, Y., Zhu, W., Gao, F., Wang, Y., et al. (2015). Type VI secretion system transports Zn<sup>2+</sup> to combat multiple stresses and host immunity. *PLoS Pathog.* 11:e1005020. doi: 10.1371/journal.ppat.1005020
- Whitney, J. C., Beck, C. M., Goo, Y. A., Russell, A. B., Harding, B. N., De Leon, J. A., et al. (2014). Genetically distinct pathways guide effector export through the type VI secretion system. *Mol. Microbiol.* 92, 529–542. doi: 10.1111/mmi.12571

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2015 Tang, Liang, Moore and Dong. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.