



Insights into the Quorum-Sensing Activity in *Aeromonas hydrophila* Strain M013 as Revealed by Whole-Genome Sequencing

Wen-Si Tan, Wai-Fong Yin, 💿 Kok-Gan Chan

Division of Genetics and Molecular Biology, Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia

Aeromonas hydrophila species can be found in warm climates and can survive in different environments. They possess the ability to communicate within their populations, which is known as quorum sensing. In this work, we present the draft genome sequence of *A. hydrophila* M013, a bacterium isolated from a Malaysian tropical rainforest waterfall.

Received 19 November 2014 Accepted 20 November 2014 Published 2 January 2015

Citation Tan W-S, Yin W-F, Chan K-G. 2015. Insights into quorum-sensing activity in Aeromonas hydrophila strain M013 as revealed by whole-genome sequencing. Genome Announc. 3(1):e01372-14. doi:10.1128/genomeA.01372-14.

Copyright © 2015 Tan et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 3.0 Unported license.

Address correspondence to Kok-Gan Chan, kokgan@um.edu.my.

For a concerted response action to occur in a bacterial population, each bacterium must be aware of and respond to others in unison (1, 2). The term "quorum sensing" (QS) was coined to explain bacterial communication in which synchronization of small diffusible molecules in a population stimulates a series of gene expressions (3). The aquatic environment provides a reservoir for microorganisms, and the microbial contamination of water often leads to major concerns (4). *Aeromonas hydrophila*, commonly known as waterborne bacteria, can cause infections, such as diarrhea, in humans, where it utilizes its QS ability to coordinate and enhance adaptation to various environments (5, 6). In this study, the *A. hydrophila* strain M013 was isolated from the Sungai Tua waterfall. In order to further explore the genetic makeup of the QS system, whole-genome sequencing was performed.

The MasterPure DNA purification kit (Epicentre, Inc., Madison, WI, USA) was used to extract the genomic DNA, while the quality of extracted DNA was examined via a NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA, USA) and a Qubit version 2.0 fluorometer (Life Technologies, Carlsbad, CA, USA). The purified DNA was subjected to whole-genome shotgun sequencing on an Illumina MiSeq personal sequencer (Illumina, Inc., San Diego, CA, USA), which generated 4,987,814 paired-end reads. The trimmed sequences (990,071 quality reads) were *de novo* assembled with CLC Genomic Workbench version 5.1 (CLC Bio, Denmark). A total of 164 contigs and an N_{50} of approximately 72,471 bp were generated.

The draft genome of the strain M013 isolate contained 4,967,716 bp, with an average coverage of 38-fold with a G+C content of 61%. The gene prediction was then performed with a prokaryote gene prediction algorithm by using Prodigal (version 2.60) (7), while rRNA and tRNA were predicted with RNAmmer (8) and tRNAscan SE version 1.21 (9), respectively. Subsequently, the M013 sequence was annotated with RAST (10). The analyses of the draft genomes identified 4,360 open reading frames (ORFs), 92 tRNAs, and one copy each of 5S rRNA, 16S rRNA, and 23S rRNA.

The complete ORFs of *A. hydrophila* strain M013 *luxI* and *luxR* homologues were predicted to be located at contig 75. This wholegenome sequence allows deeper understanding of the genetic makeup of *A. hydrophila* and may help in identifying the link between pathogenicity and virulence factors of this strain and its QS properties (5, 11).

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number JRWS00000000. The version described in this paper is the first version, JRWS01000000.

ACKNOWLEDGMENTS

This work was supported by the University of Malaya via High Impact Research grants (UM.C/625/1/HIR/MOHE/CHAN/01, grant A-000001-50001 and UM-MOHE HIR grant UM.C/625/1/HIR/MOHE/CHAN/14/1, grant H-50001-A000027) awarded to K.-G.C.

REFERENCES

- Fuqua WC, Winans SC, Greenberg EP. 1994. Quorum sensing in bacteria—the LuxR-LuxI family of cell density-responsive transcriptional regulators. J Bacteriol 176:269–275.
- 2. Williams P, Winzer K, Chan WC, Camara M. 2007. Look who's talking: communication and quorum sensing in the bacterial world. Philos Trans R Soc B 362:1119–1134. http://dx.doi.org/10.1098/rstb.2007.2039.
- Tan WS, Yunos NYM, Tan PW, Mohamad NI, Adrian T-G-S, Yin WF, Chan KG. 2014. Freshwater-borne bacteria isolated from a Malaysian rainforest waterfall exhibiting quorum sensing properties. Sensors 14: 10527–10537. http://dx.doi.org/10.3390/s140610527.
- Tan WS, Muhamad Yunos NY, Tan PW, Mohamad NI, Adrian T-G-S, Yin WF, Chan KG. 2014. *Pantoea* sp. isolated from tropical fresh water exhibiting N-acyl homoserine lactone production. Scientific World J 2014:828971. http://dx.doi.org/10.1155/2014/828971.
- Chan XY, Chua KG, Pothicheary SD, Yin WF, Chan KG. 2012. Draft genome sequence of an *Aeromonas* sp. strain 159 clinical isolate that shows quorum sensing activity. J Bacteriol 194: 6350. http://dx.doi.org/10.1128/ JB.01642-12.
- Lynch MJ, Swift S, Kirke DF, Keevil CW, Dodd CER, Williams P. 2002. The regulation of biofilm development by quorum sensing in *Aeromonas hydrophila*. Environ Microbiol 4:18–28. http://dx.doi.org/10.1046/j.1462 -2920.2002.00264.x.
- Hyatt D, Chen GL, LoCascio PF, Land ML, Larimer FW, Hauser LJ. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics 11:119. http://dx.doi.org/ 10.1186/1471-2105-11-119.
- 8. Lagesen K, Hallin P, Rødland EA, Staerfeldt HH, Rognes T, Ussery DW. 2007. RNAmmer: consistent and rapid annotation of ribosomal

RNA genes. Nucleic Acids Res 35:3100–3108. http://dx.doi.org/10.1093/ nar/gkm160.

- Lowe TM, Eddy SR. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res 25: 955–964. http://dx.doi.org/10.1093/nar/25.5.0955.
- Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T,

Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: rapid annotations using subsystems technology. BMC Genomics 9:75. http://dx.doi.org/10.1186/1471-2164-9-75.

 Chan KG, Puthucheary SD, Chan XY, Yin WF, Wong CS, See Too WS, Chua K-H. 2011. Quorum sensing in *Aeromonas* species isolated from patients in Malaysia. Curr Microbiol 62:167–172. http://dx.doi.org/ 10.1007/s00284-010-9689-z.