



Draft Genome Sequence of *Halomonas elongata* Strain K4, an Endophytic Growth-Promoting Bacterium Enhancing Salinity Tolerance *In Planta*

Feras F. Lafi,^a Juan S. Ramirez-Prado,^a Intikhab Alam,^b Vladimir B. Bajic,^b Heribert Hirt,^a DMaged M. Saad^a

King Abdullah University of Science and Technology (KAUST), Biological and Environmental Sciences and Engineering Division (BESE), Thuwal, Saudi Arabia^a; Computational Bioscience Research Center (CBRC), King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia^b

Halomonas elongata strain K4 is an endophytic bacterial strain that was isolated from roots of *Cyperus conglomeratus* collected at the Red Sea coast in Thuwal, Saudi Arabia. Here, we present a draft genome sequence of this strain, highlighting a number of pathways involved in plant growth promotion under salt stress.

Received 4 September 2016 Accepted 12 September 2016 Published 3 November 2016

Citation Lafi FF, Ramirez-Prado JS, Alam I, Bajic VB, Hirt H, Saad MM. 2016. Draft genome sequence of *Halomonas elongata* strain K4, an endophytic growth-promoting bacterium enhancing salinity tolerance *in planta*. Genome Announc 4(6):e01214-16. doi:10.1128/genomeA.01214-16.

Copyright © 2016 Lafi et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Heribert Hirt, heribert.hirt@kaust.edu.sa.

Within the framework of the Darwin21 project (http://www .darwin21.net), an extensive microbial collection of isolates from roots of different desert plants was obtained and revealed a number of strains belonging to the *Halomonadaceae* family that have the ability to promote the growth of *Arabidopsis thaliana* plants under salt stress conditions. Members of the *Halomonadaceae* family are Gram-negative, rod-shaped, and slightly or moderately halotolerant bacteria (1). They are characterized by their metabolic capacity for salt tolerance, including the synthesis of ectoine (2). Ectoine (1,4,5,6-tetrahydro-2-methyl-4pyrimidine carboxylic acid) is a widely distributed compatible solute accumulating in halophilic and halotolerant microorganisms to prevent osmotic stress in highly saline environments and is responsible for enhancing salt tolerance in bacteria (3, 4) and plants (5–7).

Halomonas elongata strain K4 was isolated from surfacesterilized roots of Cyperus conglomeratus, a naturally occurring plant in the desert of the Arabian Peninsula. The plants were collected approximately 10 m from the coast of the Red Sea near Thuwal, Saudi Arabia (22.3095°N, 39.1047°E). The root extracts were plated on R2A media (8) supplemented with 3% NaCl. Single colonies were subcultured after selection from a 10⁻⁴ dilution plate grown at 28°C. Based on 16S rRNA gene analysis, the K4 strain was closely related to both H. elongata 1H9 (NR_074782) and H. elongata DSM 2581 (NC_014532) with 99% sequence similarity (2). Genomic DNA of the K4 strain was extracted using Qiagen's DNeasy blood and tissue kit following the manufacturer's protocol. The DNA library was constructed as described previously (9) and sequenced by paired-end Illumina MiSeq. Contig assembly was done with Spades assembler version 3.6 with a 1-kb contig cutoff size (10). De novo assembly of the MiSeq reads for *H. elongata* strain K4 resulted in 35 contigs with a total length of 3,469,874 bp and a mean contig size of ~99,139 bp. The N_{50} was 272,670 bp and the L_{50} was reached by 4 contigs, with an average GC content of 63.4%. MegaBLAST searches (11) of the K4 concatenated genome against the NCBI reference genome database

(http://www.ncbi.nlm.nih.gov/genome) revealed that the closest relative genome was *H. elongata* DSM 2581 (NC_014532) with 87% sequence coverage and 95% sequence identity.

Genome annotation was carried out with the Indigo pipeline (12) with the exception of open reading frame (ORF) prediction by FragGeneScan (13). The annotation of *H. elongata* strain K4 resulted in 2,354 ORFs, 5 rRNAs, 59 tRNAs, and 39 ncRNAs. Analysis of the genome indicated the presence of a single copy of the ectABC operon (14) involved in the biosynthesis of ectoine. The genome coded for the three main enzymes involved in the ectoine pathway, namely, L-2,4-diaminobutyric acid acetyltransferase (EctA) (EC: 2.3.1.178), L-2,4-diaminobutyric acid aminotransferase (EctB) (EC: 2.6.1.76), and L-ectoine synthase (EctC) (EC: 4.2.1.108). An additional gene *ectD* coding for ectoine hydroxylase (EC: 1/14/11) is present, and this enzyme converts ectoine to hydroxyectoine (15). Furthermore, the genome encoded other osmoprotectant-related enzymes such as trehalose phosphatase (EC:3.1.3.12) (16) and mannitol-1-phosphate 5-dehydrogenase (EC:1.1.1.17) (17), suggesting a possible role for this endophyte in enhancing salinity tolerance in plants.

Accession number(s). The genome sequence of *H. elongata* strain K4 was deposited at DDBJ/EMBL/GenBank under the accession number LWGO00000000. The version described in this paper is the first version, LWGO01000000.

ACKNOWLEDGMENTS

Genome sequencing was performed at the Bioscience Core Laboratory of the King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia. We are grateful for the use of the Dragon and Snap-Dragon computer clusters at the Computational Bioscience Research Center (CBRC) of KAUST.

This work was supported by a base fund research grant to H.H. from the King Abdullah University of Science and Technology (KAUST). Computational aspects of this work have been supported by the KAUST Office of Sponsored Research (OSR) under award numbers URF/1/1976-02 and FCS/1/2448-01 to V.B.B.

FUNDING INFORMATION

This work, including the efforts of Heribert Hirt, was funded by KAUST (BAS/1/1062-01-01). This work, including the efforts of Vladimir B. Bajic, was funded by KAUST Office of Sponsored Research (URF/1/1976-02 and FCS/1/2448-01).

This work was supported by a base fund research grant to H.H. from the King Abdullah University of Science and Technology (KAUST). Computational aspects of this work have been supported by the KAUST Office of Sponsored Research (OSR) under award no. URF/1/1976-02 and FCS/1/ 2448-01 to V.B.B.

REFERENCES

- Dobson SJ, Franzmann PD. 1996. Unification of the genera *Deleya* (Baumann et al 1983), *Halomonas* (Vreeland et al 1980), and *Halovibrio* (Fendrich 1988) and the species *Paracoccus halodenitrificans* (Robinson and Gibbons 1952) into a single genus, *Halomonas*, and placement of the genus *Zymobacter* in the family *Halomonadaceae*. Int J Syst Bacteriol 46: 550–558. http://dx.doi.org/10.1099/00207713-46-2-550.
- Schwibbert K, Marin-Sanguino A, Bagyan I, Heidrich G, Lentzen G, Seitz H, Rampp M, Schuster SC, Klenk HP, Pfeiffer F, Oesterhelt D, Kunte HJ. 2011. A blueprint of ectoine metabolism from the genome of the industrial producer *Halomonas elongata* DSM 2581^T. Environ Microbiol 13:1973–1994. http://dx.doi.org/10.1111/j.1462-2920.2010.02336.x.
- 3. Galinski EA, Pfeiffer HP, Trüper HG. 1985. 1,4,5,6-tetrahydro-2methyl-4-pyrimidinecarboxylic acid: a novel cyclic amino acid from halophilic phototrophic bacteria of the genus *Ectothiorhodospira*. Eur J Biochem 149:135–139. http://dx.doi.org/10.1111/j.1432 -1033.1985.tb08903.x.
- Göller K, Ofer A, Galinski EA. 1998. Construction and characterization of an NaCl-sensitive mutant of *Halomonas elongata* impaired in ectoine biosynthesis. FEMS Microbiol Lett 161:293–300. http://dx.doi.org/ 10.1111/j.1574-6968.1998.tb12960.x.
- Nakayama H, Yoshida K, Ono H, Murooka Y, Shinmyo A. 2000. Ectoine, the compatible solute of *Halomonas elongata*, confers hyperosmotic tolerance in cultured tobacco cells. Plant Physiol 122:1239–1247. http://dx.doi.org/10.1104/pp.122.4.1239.
- Moghaieb RE, Tanaka N, Saneoka H, Murooka Y, Ono H, Morikawa H, Nakamura A, Nguyen NT, Suwa R, Fujita K. 2006. Characterization of salt tolerance in ectoine-transformed tobacco plants (*Nicotiana tabaccum*): photosynthesis, osmotic adjustment, and nitrogen partitioning. Plant Cell Environ 29:173–182. http://dx.doi.org/10.1111/j.1365 -3040.2005.01410.x.

- Moghaieb RE, Nakamura A, Saneoka H, Fujita K. 2011. Evaluation of salt tolerance in ectoine-transgenic tomato plants (*Lycopersicon esculentum*) in terms of photosynthesis, osmotic adjustment, and carbon partitioning. GM Crops 2:58–65. http://dx.doi.org/10.4161/gmcr.2.1.15831.
- Reasoner DJ, Geldreich EE. 1985. A new medium for the enumeration and subculture of bacteria from potable water. Appl Environ Microbiol 49:1–7.
- Lafi FF, Bokhari A, Alam I, Bajic VB, Hirt H, Saad MM. 2016. Draft genome sequence of the plant growth-promoting *Cupriavidus gilardii* strain JZ4 isolated from the desert plant *Tribulus terrestris*. Genome Announc 4(4):e00678-16. http://dx.doi.org/10.1128/genomeA.00678-16.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to singlecell sequencing. J Comput Biol 19:455–477. http://dx.doi.org/10.1089/ cmb.2012.0021.
- Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL. 2009. BLAST+: architecture and applications. BMC Bioinformatics 10:421. http://dx.doi.org/10.1186/1471-2105-10-421.
- Alam I, Antunes A, Kamau AA, Ba Alawi W, Kalkatawi M, Stingl U, Bajic VB. 2013. INDIGO—INtegrated data warehouse of microbial genomes with examples from the Red Sea extremophiles. PLoS One 8:e82210. http://dx.doi.org/10.1371/journal.pone.0082210.
- Rho M, Tang H, Ye Y. 2010. FragGeneScan: predicting genes in short and error-prone reads. Nucleic Acids Res 38:e191. http://dx.doi.org/10.1093/ nar/gkq747.
- 14. Zhu D, Liu J, Han R, Shen G, Long Q, Wei X, Liu D. 2014. Identification and characterization of ectoine biosynthesis genes and heterologous expression of the *ectABC* gene cluster from *Halomonas* sp. QHL1, a moderately halophilic bacterium isolated from Qinghai Lake. J Microbiol 52: 139–147. http://dx.doi.org/10.1007/s12275-014-3389-5.
- Bursy J, Pierik AJ, Pica N, Bremer E. 2007. Osmotically induced synthesis of the compatible solute hydroxyectoine is mediated by an evolutionarily conserved ectoine hydroxylase. J Biol Chem 282:31147–31155. http://dx.doi.org/10.1074/jbc.M704023200.
- Ge LF, Chao DY, Shi M, Zhu MZ, Gao JP, Lin HX. 2008. Overexpression of the trehalose-6-phosphate phosphatase gene OsTPP1 confers stress tolerance in rice and results in the activation of stress responsive genes. Planta 228:191–201. http://dx.doi.org/10.1007/s00425-008-0729-x.
- 17. Chiang YJ, Stushnoff C, McSay AE. 2005. Overexpression of mannitol-1-phosphate dehydrogenase increases mannitol accumulation and adds protection against chilling injury in petunia. J Am Soc Hort Sci 130: 605–610.