



## **Complete Genome Sequence of a New Halophilic Archaeon, Haloarcula taiwanensis, Isolated from a Solar Saltern in Southern Taiwan**

**Pei-Chun Chen,a Ting-Wen Chen,b,c Yi-An Han,a Wailap Victor Ng,d,e,f,g Chii-Shen Yanga**

aDepartment of Biochemical Science and Technology, National Taiwan University, Taipei, Taiwan

<sup>b</sup>Molecular Medicine Research Center, Chang Gung University, Guishan, Taiwan

c Bioinformatics Center, Chang Gung University, Guishan, Taiwan

<sup>d</sup>Department of Biotechnology and Laboratory Science in Medicine, National Yang Ming University, Taipei, Taiwan

eInstitute of Biomedical Informatics, National Yang Ming University, Taipei, Taiwan

f Center for Systems and Synthetic Biology, National Yang Ming University, Taipei, Taiwan

<sup>g</sup>Department of Biochemistry, Kaohsiung Medical University, Kaohsiung, Taiwan

**ABSTRACT** We report here the completion of the genome sequence of a new species of haloarchaea, Haloarcula taiwanensis, isolated in southern Taiwan. The 3,721,706-bp genome consisted of chromosome I (2,966,258 bp, 63.6% GC content), chromosome II (525,233 bp, 59.6% GC content), plasmid pNYT1 (129,893 bp, 55.3% GC content), and plasmid pNYT2 (100,322 bp, 55.7% GC content).

**Haloarchaea** (halophilic archaea) are prokaryotes that thrive in high-salt aqueous<br>**Environments, such as solar salterns and salt lakes**. They adopt a microbial rhodopsin (M-Rho) system capable of exerting light-driven ion transportation and photosensing to assist in solar energy harvest, and they adjust their physical position with illumination that favors their optimal survival [\(1,](#page-1-0) [2\)](#page-1-1). Among the M-Rho proteins, bacteriorhodopsin is a light-driven outward proton pump that can generate a proton gradient for further ATP generation via F1Fo ATP synthase [\(3,](#page-1-2) [4\)](#page-1-3), while halorhodopsin functions as a light-driven inward chloride pump [\(5\)](#page-1-4) to maintain, at least, the osmolarity of cells. Three types of sensory rhodopsins (SR) have been identified, i.e., SRI [\(6,](#page-1-5) [7\)](#page-1-6) and SRII [\(8\)](#page-1-7), which mediate positive and negative phototaxis responses, respectively, and SRM [\(9\)](#page-1-8), which senses green light, although its function has yet to be elucidated.

Crystallographic studies unveiled the structures of bacteriorhodopsins [\(10,](#page-1-9) [11\)](#page-1-10), halorhodopsins [\(12,](#page-1-11) [13\)](#page-1-12), and a sensory rhodopsin II (NpSRII) from Natronomonas pharaonis [\(14\)](#page-1-13). Both NpSRII alone and NpSRII-NpHtrII signal transducer structures were resolved, and the signal transduction from photoreceptor to transducer was proposed [\(15\)](#page-1-14). On the other hand, possibly due to instability under low-salt conditions, no SRI protein structure has been reported previously. To search for stable SRI protein candidates, we sequenced a new Haloarcula species, Haloarcula taiwanensis, isolated in southern Taiwan, and found that it possessed a four-rhodopsin system, including the SRI.

Whole-genome shotgun sequences were obtained using a PacBio single-molecule real-time (SMRT) sequencer [\(16\)](#page-1-15) from Genomics BioSci & Tech (Taipei, Taiwan). The shotgun sequences were assembled using the Hierarchical Genome Assembly Process 3 (HGAP 3) software [\(17\)](#page-1-16). The assembled contigs contained redundant terminal repeated sequences (RTRS), which were detected by Blast2seq analysis of each assembled Haloarcula taiwanensis contig sequence against itself. Within the 17,951- to 55,432-bp imperfect RTRS of the four contigs, multiple polymorphic sites, usually single nucleotide

**Received** 11 December 2017 **Accepted** 21 December 2017 **Published** 1 February 2018 **Citation** Chen P-C, Chen T-W, Han Y-A, Ng WV, Yang C-S. 2018. Complete genome sequence of a new halophilic archaeon, Haloarcula taiwanensis, isolated from a solar saltern in southern Taiwan. Genome Announc 6:e01529-17. [https://doi.org/](https://doi.org/10.1128/genomeA.01529-17) [10.1128/genomeA.01529-17.](https://doi.org/10.1128/genomeA.01529-17)

**Copyright** © 2018 Chen et al. This is an openaccess article distributed under the terms of the [Creative Commons Attribution 4.0](https://creativecommons.org/licenses/by/4.0/) [International license.](https://creativecommons.org/licenses/by/4.0/)

Address correspondence to Wailap Victor Ng, [wvng@ym.edu.tw,](mailto:wvng@ym.edu.tw) or Chii-Shen Yang, [chiishen@ntu.edu.tw.](mailto:chiishen@ntu.edu.tw)

P.-C.C. and T.-W.C. contributed equally to this work.

W.V.N. and C.-S.Y. share senior authorship.

insertions/deletions, were detected. To identify the likely correct sequence, each pair of approximately 240-bp sequences with the polymorphic site near the center were searched against archaeal reference protein sequences using the Blastx algorithm, and the one which matched to protein sequence(s) was considered to have the correct sequence [\(18\)](#page-1-17). Finally, one of the RTRS copies in each contig was removed by splicing the likely correct sequence in the terminal repeats using the EditPad Lite 7 text editor (Just Great Software).

**Accession number(s).** The sequences of chromosome I, chromosome II, pNYT1, and pNYT2 have been deposited in GenBank under the accession numbers [CP019154,](https://www.ncbi.nlm.nih.gov/nuccore/CP019154) [CP019155,](https://www.ncbi.nlm.nih.gov/nuccore/CP019155) [CP019156,](https://www.ncbi.nlm.nih.gov/nuccore/CP019156) and [CP019157,](https://www.ncbi.nlm.nih.gov/nuccore/CP019157) respectively.

## **ACKNOWLEDGMENTS**

This work was supported by grants from the Ministry of Science and Technology to C.-S. Yang (grant 105-2311-B-002-036) and to W. V. Ng (grant 105-2320-B-010-020-MY3).

## <span id="page-1-0"></span>**REFERENCES**

- 1. Hildebrand E, Dencher N. 1975. Two photosystems controlling behavioural responses of Halobacterium halobium. Nature 257:46-48. [https://](https://doi.org/10.1038/257046a0) [doi.org/10.1038/257046a0.](https://doi.org/10.1038/257046a0)
- <span id="page-1-2"></span><span id="page-1-1"></span>2. Spudich JL. 2006. The multitalented microbial sensory rhodopsins. Trends Microbiol 14:480 – 487. [https://doi.org/10.1016/j.tim.2006.09.005.](https://doi.org/10.1016/j.tim.2006.09.005)
- 3. Kayushin LP, Skulachev VP. 1974. Bacteriorhodopsin as an electrogenic proton pump: reconstitution of bacteriorhodopsin proteoliposomes generating delta psi and delta pH. FEBS Lett 39:39 – 42. [https://doi.org/](https://doi.org/10.1016/0014-5793(74)80011-6) [10.1016/0014-5793\(74\)80011-6.](https://doi.org/10.1016/0014-5793(74)80011-6)
- <span id="page-1-3"></span>4. Oesterhelt D. 1975. The purple membrane of Halobacterium halobium: a new system for light energy conversion. Ciba Found Symp Chapter 9:147–167. [https://doi.org/10.1002/9780470720134.ch9.](https://doi.org/10.1002/9780470720134.ch9)
- <span id="page-1-5"></span><span id="page-1-4"></span>5. Schobert B, Lanyi JK. 1982. Halorhodopsin is a light-driven chloride pump. J Biol Chem 257:10306 –10313.
- 6. Bogomolni RA, Spudich JL. 1982. Identification of a third rhodopsin-like pigment in phototactic Halobacterium halobium. Proc Natl Acad Sci U S A 79:6250 – 6254. [https://doi.org/10.1073/pnas.79.20.6250.](https://doi.org/10.1073/pnas.79.20.6250)
- <span id="page-1-6"></span>7. Spudich JL, Bogomolni RA. 1984. Mechanism of colour discrimination by a bacterial sensory rhodopsin. Nature 312:509 –513. [https://doi.org/10](https://doi.org/10.1038/312509a0) [.1038/312509a0.](https://doi.org/10.1038/312509a0)
- <span id="page-1-7"></span>8. Takahashi T, Mochizuki Y, Kamo N, Kobatake Y. 1985. Evidence that the long-lifetime photointermediate of s-rhodopsin is a receptor for negative phototaxis in Halobacterium halobium. Biochem Biophys Res Commun 127:99 –105. [https://doi.org/10.1016/S0006-291X\(85\)80131-5.](https://doi.org/10.1016/S0006-291X(85)80131-5)
- <span id="page-1-8"></span>9. Fu HY, Lin YC, Chang YN, Tseng H, Huang CC, Liu KC, Huang CS, Su CW, Weng RR, Lee YY, Ng WV, Yang CS. 2010. A novel six-rhodopsin system in a single archaeon. J Bacteriol 192:5866 –5873. [https://doi.org/10.1128/](https://doi.org/10.1128/JB.00642-10) [JB.00642-10.](https://doi.org/10.1128/JB.00642-10)
- <span id="page-1-10"></span><span id="page-1-9"></span>10. Henderson R. 1975. The structure of the purple membrane from Halobacterium halobium: analysis of the X-ray diffraction pattern. J Mol Biol 93:123–138. [https://doi.org/10.1016/0022-2836\(75\)90123-0.](https://doi.org/10.1016/0022-2836(75)90123-0)
- <span id="page-1-11"></span>11. Blaurock AE, Stoeckenius W. 1971. Structure of the purple membrane. Nat New Biol 233:152–155. [https://doi.org/10.1038/newbio233152a0.](https://doi.org/10.1038/newbio233152a0)
- 12. Dorset DL. 1995. Direct structure analysis in protein electron

crystallography: crystallographic phases for halorhodopsin to 6-A resolution. Proc Natl Acad Sci U S A 92:10074-10078. [https://doi.org/10.1073/](https://doi.org/10.1073/pnas.92.22.10074) [pnas.92.22.10074.](https://doi.org/10.1073/pnas.92.22.10074)

- <span id="page-1-12"></span>13. Kolbe M, Besir H, Essen LO, Oesterhelt D. 2000. Structure of the lightdriven chloride pump halorhodopsin at 1.8 A resolution. Science 288: 1390 –1396. [https://doi.org/10.1126/science.288.5470.1390.](https://doi.org/10.1126/science.288.5470.1390)
- <span id="page-1-13"></span>14. Iwamoto M, Sudo Y, Shimono K, Araiso T, Kamo N. 2005. Correlation of the O-intermediate rate with the pKa of Asp-75 in the dark, the counterion of the Schiff base of Pharaonis phoborhodopsin (sensory rhodopsin II). Biophys J 88:1215–1223. [https://doi.org/10.1529/biophysj.104](https://doi.org/10.1529/biophysj.104.045583) [.045583.](https://doi.org/10.1529/biophysj.104.045583)
- <span id="page-1-14"></span>15. Moukhametzianov R, Klare JP, Efremov R, Baeken C, Göppner A, Labahn J, Engelhard M, Büldt G, Gordeliy VI. 2006. Development of the signal in sensory rhodopsin and its transfer to the cognate transducer. Nature 440:115–119. [https://doi.org/10.1038/nature04520.](https://doi.org/10.1038/nature04520)
- <span id="page-1-15"></span>16. Eid J, Fehr A, Gray J, Luong K, Lyle J, Otto G, Peluso P, Rank D, Baybayan P, Bettman B, Bibillo A, Bjornson K, Chaudhuri B, Christians F, Cicero R, Clark S, Dalal R, Dewinter A, Dixon J, Foquet M, Gaertner A, Hardenbol P, Heiner C, Hester K, Holden D, Kearns G, Kong X, Kuse R, Lacroix Y, Lin S, Lundquist P, Ma C, Marks P, Maxham M, Murphy D, Park I, Pham T, Phillips M, Roy J, Sebra R, Shen G, Sorenson J, Tomaney A, Travers K, Trulson M, Vieceli J, Wegener J, Wu D, Yang A, Zaccarin D, Zhao P, Zhong F, Korlach J, Turner S. 2009. Real-time DNA sequencing from single polymerase molecules. Science 323:133–138. [https://doi.org/10.1126/](https://doi.org/10.1126/science.1162986) [science.1162986.](https://doi.org/10.1126/science.1162986)
- <span id="page-1-16"></span>17. Chin CS, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J. 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. Nat Methods 10:563–569. [https://doi.org/10.1038/](https://doi.org/10.1038/nmeth.2474) [nmeth.2474.](https://doi.org/10.1038/nmeth.2474)
- <span id="page-1-17"></span>18. McGinnis S, Madden TL. 2004. BLAST: at the core of a powerful and diverse set of sequence analysis tools. Nucleic Acids Res 32:W20 –W25. [https://doi.org/10.1093/nar/gkh435.](https://doi.org/10.1093/nar/gkh435)