

# Draft Genome Sequence of the *Sulfolobales* Archaeon AZ1, Obtained through Metagenomic Analysis of a Mexican Hot Spring

Luis E. Servín-Garcidueñas, Esperanza Martínez-Romero

Center for Genomic Sciences, Department of Ecological Genomics, National University of Mexico, Cuernavaca, Morelos, Mexico

The *Sulfolobales* archaea have been found inhabiting acidic hot springs all over the world. Here, we report the 1.798-Mbp draft genome sequence of the thermoacidophilic *Sulfolobales* archaeon AZ1, reconstructed from the metagenome of a Mexican hot spring. Sequence-based comparisons revealed that the *Sulfolobales* archaeon AZ1 represents a novel candidate genus.

Received 10 February 2014 Accepted 13 February 2014 Published 6 March 2014

Citation Servín-Garcidueñas LE, Martínez-Romero E. 2014. Draft genome sequence of the *Sulfolobales* archaeon AZ1, obtained through metagenomic analysis of a Mexican hot spring. *Genome Announc.* 2(2):e00164-14. doi:10.1128/genomeA.00164-14.

Copyright © 2014 Servín-Garcidueñas and Martínez-Romero. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](http://creativecommons.org/licenses/by/nd/3.0/).

Address correspondence to Luis E. Servín-Garcidueñas, luis.e.servin@gmail.com.

The order *Sulfolobales* is placed within the phylum *Crenarchaeota* (1), and some of its species are considered model organisms (2). The order *Sulfolobales* comprises the genera *Sulfolobus*, *Acidianus*, *Metallosphaera*, *Stygiolobus*, and *Sulfurisphaera* (3–7). *Sulfolobus* contains the highest number of sequenced strains (8–15), and until now, only one complete *Acidianus* genome sequence was available (16). *Metallosphaera* contains three sequenced species (17, 18, 19), including “*Metallosphaera yellowstonensis*,” which was first described through metagenomic efforts (20). A novel *Sulfolobales* archaeon has also been discovered from a metagenomic study (21). We report the draft metagenomic sequence of the *Sulfolobales* archaeon AZ1, the first member of the “*Candidatus Aramenus*” genus.

Samples were collected from a hot spring (pH 3.6 and 65°C) located at Los Azufres National Park, Mexico, during March 2009. Environmental DNA was purified using the Ultra-Clean microbial DNA and the Ultra-Clean mega soil DNA kits (MoBio Laboratories, Inc., Carlsbad and Solana Beach, CA). Sequencing was performed with an Illumina-GAIIX platform, producing 36-bp paired-end reads with 300-bp inserts representing 216 Mbp. Reads were assembled *de novo* using Velvet version 1.2.10 (22). A total of 163 contigs were verified by BLASTN searches to be of archaeal origin. All other contigs were assembled into the *Sulfolobales* Mexican rudivirus 1 (SMR1) (23) and the *Sulfolobales* Mexican fusellovirus 1 (SMF1) (24) sequences. All reads were mapped to the archaeal contigs using Maq 0.7.1 (25). The mapping reads were reassembled to eliminate gaps from the archaeal contigs by sequence extensions. Genome annotation was performed with the NCBI Prokaryotic Genomes Automatic Annotation Pipeline version 2.0 ([https://www.ncbi.nlm.nih.gov/genome/annotation\\_prok/](https://www.ncbi.nlm.nih.gov/genome/annotation_prok/)). DNA-DNA hybridization (DDH) values were computed using the Genome-to-Genome Distance Calculator (26, 27) version 2.0 (28).

The metagenome assembly was 1,798,894 bp, and only one type of 16S rRNA gene was detected. We retrieved a consensus genome of a population consisting of a dominant *Sulfolobales* archaeon that was designated AZ1. The consensus genome contains

46 contigs with a coverage of 71.9× and an  $N_{50}$  value of 223,688 bp. A total of 2,002 genes were predicted, including 1,975 protein-coding genes. The consensus genome had a G+C content of 47%, higher than the 34.1% of the *Acidianus hospitalis* W1 genome and the 32.8 to 36.7% of the *Sulfolobus* genomes. The G+C content more closely resembles the 42 to 47.7% G+C contents of *Metallosphaera* genomes.

The 16S rRNA gene from the archaeon AZ1 shares 93% sequence identity with the corresponding gene from *A. hospitalis* W1. A 95% sequence identity has been proposed as a reasonable value to limit different genera (29, 30). Genome sequence comparisons between the archaeon AZ1 and *A. hospitalis* W1 revealed a DDH estimate of 16.10%, well below the 70% proposed for species definition (30, 31). The archaeon AZ1 would then correspond to a novel *Sulfolobales* genus. The name “*Candidatus Aramenus sulfurataquae*,” meaning “the guardian of the sulfurated water,” is proposed. The word “Arameni,” from the Mexican Purepecha language, means “guardian/custodian of the water.” Further characterization of the “*Candidatus Aramenus*” genus is in progress.

**Nucleotide sequence accession number.** This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. [ASRH00000000](https://www.ncbi.nlm.nih.gov/nuccore/ASRH00000000).

## ACKNOWLEDGMENTS

This research was supported by PAPIIT IN205412 from DGAPA, UNAM, and SUBNARGEM, SAGARPA. L.E.S.-G. received a Ph.D. scholarship from CONACYT (Mexico).

We thank Jean P. Euzéby (nomenclature reviewer of the *International Journal of Systematic and Evolutionary Microbiology*) for reviewing scientific names. We also thank the Programa de Doctorado en Ciencias Biomédicas from UNAM. We thank the UUSM from UNAM for sample sequencing. The Comisión Federal de Electricidad personnel provided a permit for sampling. We thank Jesús Campos García from the UMSNH for providing laboratory facilities. Samplings were carried out with the efforts of Jonathan Lopez, José Luis Servín, and Cecilia Garcidueñas.

## REFERENCES

1. Stetter KO, López-Casillas F, Bai DH, Luo X, Pape ME. 1989. Order III. *Sulfolobales* ord. nov. family *Sulfolobaceae* fam. nov., p 2250–2251. In Staley JT, Bryant MP, Pfennig N, Holt JG, López-Casillas F, Bai DH, Luo X, Pape ME (ed), *Bergey's manual of systematic bacteriology*, vol 3, 1st ed. Williams & Wilkins, Baltimore, MD.
2. Leigh JA, Albers SV, Atomi H, Allers T. 2011. Model organisms for genetics in the domain Archaea: methanogens, halophiles, *Thermococcales* and *Sulfolobales*. *FEMS Microbiol. Rev.* 35:577–608. <http://dx.doi.org/10.1111/j.1574-6976.2011.00265.x>.
3. Brock TD, Brock KM, Belly RT, Weiss RL. 1972. *Sulfolobus*: a new genus of sulfur-oxidizing bacteria living at low pH and high temperature. *Arch. Mikrobiol.* 84:54–68. <http://dx.doi.org/10.1007/BF00408082>.
4. Segerer A, Neuner AM, Kristjansson JK, Stetter KO. 1986. *Acidianus infernus* gen. nov., sp. nov., and *Acidianus brierleyi* comb. nov.: facultatively aerobic, extremely acidophilic thermophilic sulfur-metabolizing archaeabacteria. *Int. J. Syst. Bacteriol.* 36:559–564. <http://dx.doi.org/10.1099/00207713-36-4-559>.
5. Huber G, Spinnler C, Gambacorta A, Stetter KO. 1989. *Metallosphaera sedula* gen. nov. and sp. nov. represents a new genus of aerobic, metal-mobilizing, thermoacidophilic archaeabacteria. *Syst. Appl. Microbiol.* 12: 38–47. [http://dx.doi.org/10.1016/S0723-2020\(89\)80038-4](http://dx.doi.org/10.1016/S0723-2020(89)80038-4).
6. Segerer AH, Trincone A, Gahrtz M, Stetter KO. 1991. *Stygiolobus azoricus* gen. nov., sp. nov. represents a novel genus of anaerobic, extremely thermoacidophilic archaeabacteria of the order *Sulfolobales*. *Int. J. Syst. Bacteriol.* 41:495–501. <http://dx.doi.org/10.1099/00207713-41-4-495>.
7. Kurosawa N, Itoh YH, Iwai T, Sugai A, Uda I, Kimura N, Horiuchi T, Itoh T. 1998. *Sulfurisphaera ohwakuenensis* gen. nov., sp. nov., a novel extremely thermophilic acidophile of the order *Sulfolobales*. *Int. J. Syst. Bacteriol.* 48(Pt 2):451–456. <http://dx.doi.org/10.1099/00207713-48-2-451>.
8. She Q, Singh RK, Confalonieri F, Zivanovic Y, Allard G, Awayez MJ, Chan-Weiher CC, Clausen IG, Curtis BA, De Moors A, Erauso G, Fletcher C, Gordon PM, Heikamp-de Jong I, Jeffries AC, Kozena CJ, Medina N, Peng X, Thi-Ngoc HP, Redder P, Schenk ME, Theriault C, Tolstrup N, Charlebois RL, Doolittle WF, Duguet M, Gaasterland T, Garrett RA, Ragan MA, Sensen CW, Van der Oost J. 2001. The complete genome of the crenarchaeon *Sulfolobus solfataricus* P2. *Proc. Natl. Acad. Sci. U. S. A.* 98:7835–7840. <http://dx.doi.org/10.1073/pnas.141222098>.
9. Kawarabayasi Y, Hino Y, Horikawa H, Jin-no K, Takahashi M, Sekine M, Baba S, Ankai A, Kosugi H, Hosoyama A, Fukui S, Nagai Y, Nishijima K, Otsuka R, Nakazawa H, Takamiya M, Kato Y, Yoshizawa T, Tanaka T, Kudoh Y, Yamazaki J, Kushida N, Oguchi A, Aoki K, Masuda S, Yanagii M, Nishimura M, Yamagishi A, Oshima T, Kikuchi H. 2001. Complete genome sequence of an aerobic thermoacidophilic crenarchaeon, *Sulfolobus tokodaii* strain 7. *DNA Res.* 8:123–140. <http://dx.doi.org/10.1093/dnarese.8.4.123>.
10. Chen L, Brügger K, Skovgaard M, Redder P, She Q, Torarinsson E, Greve B, Awayez M, Zibat A, Klenk HP, Garrett RA. 2005. The genome of *Sulfolobus acidocaldarius*, a model organism of the *Crenarchaeota*. *J. Bacteriol.* 187:4992–4999. <http://dx.doi.org/10.1128/JB.187.14.4992-499.2005>.
11. Reno ML, Held NL, Fields CJ, Burke PV, Whitaker RJ. 2009. Biogeography of the *Sulfolobus islandicus* pan-genome. *Proc. Natl. Acad. Sci. U. S. A.* 106:8605–8610. <http://dx.doi.org/10.1073/pnas.0808945106>.
12. Guo L, Brügger K, Liu C, Shah SA, Zheng H, Zhu Y, Wang S, Lillestøl RK, Chen L, Frank J, Prangishvili D, Paulin L, She Q, Huang L, Garrett RA. 2011. Genome analyses of Icelandic strains of *Sulfolobus islandicus*, model organisms for genetic and virus-host interaction studies. *J. Bacteriol.* 193:1672–1680. <http://dx.doi.org/10.1128/JB.01487-10>.
13. Cadillo-Quiroz H, Didelot X, Held NL, Herrera A, Darling A, Reno ML, Krause DJ, Whitaker RJ. 2012. Patterns of gene flow define species of thermophilic Archaea. *PLoS Biol.* 10:e1001265. <http://dx.doi.org/10.1371/journal.pbio.1001265>.
14. Mao D, Grogan D. 2012. Genomic evidence of rapid, global-scale gene flow in a *Sulfolobus* species. *ISME J.* 6:1613–1616. <http://dx.doi.org/10.1038/ismej.2012.20>.
15. Jaubert C, Danioux C, Oberto J, Cortez D, Bize A, Krupovic M, She Q, Forterre P, Prangishvili D, Sezonov G. 2013. Genomics and genetics of *Sulfolobus islandicus* LAL14/1, a model hyperthermophilic archaeon. *Open Biol.* 3:130010. <http://dx.doi.org/10.1098/rsob.130010>.
16. You XY, Liu C, Wang SY, Jiang CY, Shah SA, Prangishvili D, She Q, Liu SJ, Garrett RA. 2011. Genomic analysis of *Acidianus hospitalis* W1 a host for studying crenarchaeal virus and plasmid life cycles. *Extremophiles* 15:487–497. <http://dx.doi.org/10.1007/s00792-011-0379-y>.
17. Auernik KS, Maezato Y, Blum PH, Kelly RM. 2008. The genome sequence of the metal-mobilizing, extremely thermoacidophilic archaeon *Metallosphaera sedula* provides insights into bioleaching-associated metabolism. *Appl. Environ. Microbiol.* 74:682–692. <http://dx.doi.org/10.1128/AEM.02019-07>.
18. Liu LJ, You XY, Zheng H, Wang S, Jiang CY, Liu SJ. 2011. Complete genome sequence of *Metallosphaera cuprina*, a metal sulfide-oxidizing archaeon from a hot spring. *J. Bacteriol.* 193:3387–3388. <http://dx.doi.org/10.1128/JB.05038-11>.
19. Kozubal MA, Dlakic M, Macur RE, Inskeep WP. 2011. Terminal oxidase diversity and function in “*Metallosphaera yellowstonensis*”: gene expression and protein modeling suggest mechanisms of Fe(II) oxidation in the *Sulfolobales*. *Appl. Environ. Microbiol.* 77:1844–1853. <http://dx.doi.org/10.1128/AEM.01646-10>.
20. Inskeep WP, Rusch DB, Jay ZJ, Herrgard MJ, Kozubal MA, Richardson TH, Macur RE, Hamamura N, Jennings Rd, Fouke BW, Reysenbach AL, Roberto F, Young M, Schwartz A, Boyd ES, Badger JH, Mathur EJ, Ortmann AC, Bateson M, Geesey G, Frazier M. 2010. Metagenomes from high-temperature chemotrophic systems reveal geochemical controls on microbial community structure and function. *PLoS One* 5:e9773. <http://dx.doi.org/10.1371/journal.pone.0009773>.
21. Podar M, Makarova KS, Graham DE, Wolf YI, Koonin EV, Reysenbach AL. 2013. Insights into archaeal evolution and symbiosis from the genomes of a nanoarchaeon and its inferred crenarchaeal host from Obsidian Pool, Yellowstone National Park. *Biol. Direct* 8:9. <http://dx.doi.org/10.1186/1745-6150-8-9>.
22. Zerbino DR, Birney E. 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. *Genome Res.* 18:821–829. <http://dx.doi.org/10.1101/gr.074492.107>.
23. Servín-Garcidueñas LE, Peng X, Garrett RA, Martínez-Romero E. 2013. Genome sequence of a novel archaeal ravidivirus recovered from a Mexican hot spring. *Genome Announc.* 1(1):e00040-12. <http://dx.doi.org/10.1128/genomeA.00040-12>.
24. Servín-Garcidueñas LE, Peng X, Garrett RA, Martínez-Romero E. 2013. Genome sequence of a novel archaeal fusellovirus assembled from the metagenome of a Mexican hot spring. *Genome Announc.* 1(2):e00164-13. <http://dx.doi.org/10.1128/genomeA.00164-13>.
25. Li H, Ruan J, Durbin R. 2008. Mapping short DNA sequencing reads and calling variants using mapping quality scores. *Genome Res.* 18:1851–1858. <http://dx.doi.org/10.1101/gr.078212.108>.
26. Auch AF, Klenk HP, Göker M. 2010. Standard operating procedure for calculating genome-to-genome distances based on high-scoring segment pairs. *Stand. Genomic Sci.* 2:142–148. <http://dx.doi.org/10.4056/sigs.541628>.
27. Auch AF, von Jan M, Klenk HP, Göker M. 2010. Digital DNA-DNA hybridization for microbial species delineation by means of genome-to-genome sequence comparison. *Stand. Genomic Sci.* 2:117–134. <http://dx.doi.org/10.4056/sigs.531120>.
28. Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M. 2013. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 14:60. <http://dx.doi.org/10.1186/1471-2105-14-60>.
29. Yarza P, Richter M, Peplies J, Euzeby J, Amann R, Schleifer KH, Ludwig W, Glöckner FO, Rosselló-Móra R. 2008. The all-species living tree project: a 16S rRNA-based phylogenetic tree of all sequenced type strains. *Syst. Appl. Microbiol.* 31:241–250. <http://dx.doi.org/10.1016/j.syapm.2008.07.001>.
30. Tindall BJ, Rosselló-Móra R, Busse HJ, Ludwig W, Kämpfer P. 2010. Notes on the characterization of prokaryote strains for taxonomic purposes. *Int. J. Syst. Evol. Microbiol.* 60:249–266. <http://dx.doi.org/10.1099/ijs.0.016949-0>.
31. Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O, Krichesky MI, Moore LH, Moore WEC, Murray RGE, Stackebrandt E, Starr MP, Truper HG. 1987. Report of the *ad hoc* committee on reconciliation of approaches to bacterial systematics. *Int. J. Syst. Bacteriol.* 37: 463–464. <http://dx.doi.org/10.1099/00207713-37-4-463>.