

Draft Genome of *Spiribacter salinus* M19-40, an Abundant Gammaproteobacterium in Aquatic Hypersaline Environments

Maria Jose Leon,^a Rohit Ghai,^b Ana Beatriz Fernandez,^a Cristina Sanchez-Porro,^a Francisco Rodriguez-Valera,^b Antonio Ventosa^a

Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Sevilla, Sevilla, Spain^a; Evolutionary Genomics Group, Departamento de Producción Vegetal y Microbiología, Universidad Miguel Hernández, San Juan de Alicante, Alicante, Spain^b

We have previously used a *de novo* metagenomic assembly approach to describe the presence of an abundant gammaproteobacterium comprising nearly 15% of the microbial community in an intermediate salinity solar saltern pond. We have obtained this microbe in pure culture and describe the genome sequencing of the halophilic photoheterotrophic microbe, *Spiribacter salinus* M19-40.

Received 12 December 2012 Accepted 18 December 2012 Published 14 February 2013

Citation Leon MJ, Ghai R, Fernandez AB, Sanchez-Porro C, Rodriguez-Valera F, Ventosa A. 2013. Draft genome of *Spiribacter salinus* M19-40, an abundant gammaproteobacterium in aquatic hypersaline environments. *Genome Announc.* 1(1):e00179-12. doi:10.1128/genomeA.00179-12.

Copyright © 2013 Leon et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](https://creativecommons.org/licenses/by/3.0/).

Address correspondence to Rohit Ghai, rghai@umh.es.

Hypersaline systems, e.g., solar salterns, are extreme environments of salinity higher than that of seawater. Microbes adapt to high salinities using two strategies. In the salt-out strategy, cells accumulate compatible solutes (e.g., ectoine and betaine) in the cytoplasm to exclude inorganic salts (mostly Na⁺). However, in the salt-in strategy, they actively maintain a high internal ionic concentration of potassium ions and have a significantly higher proportion of positively charged amino acids (arginine and lysine) in their proteins to maintain solubility. A range of metagenomic data sets from different salinities has been analyzed previously, and novel microbes were identified using a *de novo* metagenomic assembly approach (1). In that previous work, in a metagenomic data set from a solar saltern pond of 19% salinity, metagenomic assembly yielded 15 contigs (a total of 94 kb; G+C content, 63% to 67%) that could clearly be attributed to a gammaproteobacterium related to the genera *Alkalilimnicola* and *Nitrococcus*. However, due to the lack of a complete genome, not many observations could be made, except that the isoelectric points of the predicted proteins suggested that the microbe uses a salt-out strategy.

In an effort to culture these novel microbes, we undertook further sampling and culturing from the same saltern (Santa Pola, Spain) and from another with similar salinity (Isla Cristina, Spain), using 16S rRNA PCR for colony identification. A slow growing microbe, with a 16S rRNA sequence related to *Alkalilimnicola*, was obtained from the Isla Cristina saltern. In liquid culture, the cells appeared as spiral rods and clustered into aggregates, likely due to polysaccharide production. On solid medium, the cells appeared as slender small rods. We have named this microbe *Spiribacter salinus* M19-40, and a formal taxonomic description is in progress.

Whole-genome shotgun (WGS) sequencing of *S. salinus* M19-40 was performed using Illumina HiSeq 2× 100-bp paired-end (PE) reads and Pacific Biosciences 3- to 5-kb reads. Illumina reads at 100× coverage and long PacBio error-corrected reads (2) at a coverage of ~50× were assembled using MIRA (3) into 6

contigs (N₅₀, 1,369,469). The total genome size was 1.74 Mb (1,742,247 bp), with a G+C content of 62.65%. We identified 1,706 protein-coding genes (4) and a single rRNA operon (5). Forty-five tRNAs for all canonical 20 amino acids were identified (6). Protein-coding genes were compared to NCBI nonredundant (NR) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases for functional annotation.

Analysis of the global predicted proteome pI, and the presence of ectoine biosynthesis genes, confirm that *S. salinus* appears to have a salt-out strategy. No flagellar genes could be found, indicating the absence of swimming motility. The presence of a rhodopsin gene indicated that *S. salinus* is probably a photoheterotroph. The most similar 16S rRNA gene sequences in the Ribosomal Database Project (7) appeared to be uncultured *Ectothiorhodospiraceae* bacteria and *Alkalilimnicola* species. The identity similarity of the single 16S rRNA gene to that of the *Alkalilimnicola* 16S rRNA gene was 95%. However, the average nucleotide identity between the genomes was only 67.92%, which is indicative of the microbe belonging to a new genus.

Nucleotide sequence accession numbers. This Whole Genome Shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. ANKZ00000000. The version described in this article is the first version, ANKZ01000000.

ACKNOWLEDGMENTS

This work was supported by projects MAGYK (BIO2008-02444), Microgen (Programa CONSOLIDER-INGENIO 2010 CDS2009-00006), CGL2009-12651-C02-01, and CGL2010-19303 from the Spanish Ministerio de Ciencia e Innovación; DIMEGEN (PROMETEO/2010/089), and ACOMP/2009/155 from the Generalitat Valenciana; P10-CVI-6226 from the Junta de Andalucía; DEB-0919290 from the National Science Foundation; and MaCuMBA Project 311975 of the European Commission FP7. FEDER funds supported this project.

R.G. was supported by a Juan de la Cierva scholarship from the Spanish Ministerio de Ciencia e Innovación.

REFERENCES

1. Ghai R, Pašić L, Fernández AB, Martín-Cuadrado AB, Mizuno CM, McMahon KD, Papke RT, Stepanauskas R, Rodríguez-Brito B, Rohwer F, Sánchez-Porro C, Ventosa A, Rodríguez-Valera F. 2011. New abundant microbial groups in aquatic hypersaline environments. *Sci. Rep.* 1:135.
2. Koren S, Schatz MC, Walenz BP, Martin J, Howard JT, Ganapathy G, Wang Z, Rasko DA, McCombie WR, Jarvis ED, Phillippy AM. 2012. Hybrid error correction and *de novo* assembly of single-molecule sequencing reads. *Nat. Biotechnol.* 30:693–700.
3. Chevreur B. 2005. MIRA: an automated genome and EST assembler, p 45–46. *In* Computer science and biology: proceedings of the German conference on bioinformatics (GCB)99. Ruprecht-Karls University, Heidelberg, Germany.
4. 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.
5. 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.
6. 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.
7. Cole JR, Wang Q, Cardenas E, Fish J, Chai B, Farris RJ, Kulam-Syed-Mohideen AS, McGarrell DM, Marsh T, Garrity GM, Tiedje JM. 2009. The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. *Nucleic Acids Res.* 37:D141–D145.