Draft Genome Sequences of Kosmotoga sp. Strain DU53 and Kosmotoga arenicorallina S304

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Here, we announce the draft genome sequences of two thermophilic Thermotogae bacteria: Kosmotoga sp. strain DU53, isolated from a continental oil reservoir, and Kosmotoga arenicorallina, isolated from hydrothermal sediments. The sequences will provide further insight into evolution of the Kosmotogales.

Members of the genus Kosmotoga are anaerobic thermophilic bacteria isolated from oil reservoirs and hydrothermal environments (1, 2). The type species, Kosmotoga olearia, has an extraordinary wide growth temperature range of 20 to 79°C, and its genome was previously sequenced (3). The closest relative of Kosmotoga is the only mesophilic Thermotogae lineage, Mesotoga (4). This, together with its wide temperature growth range, makes these bacteria well suited for studying thermal adaptations (5).

Here, we present draft genome sequences of Kosmotoga arenicorallina S304 and Kosmotoga sp. strain DU53. K. arenicorallina S304 was isolated from hydrothermal sediments with a temperature of ~40°C (2) and purchased from DSMZ (https://www.dsmz.de/). Kosmotoga sp. DU53 was isolated from free-water-knockout (FWKO) water collected from oil field D (in situ) in Alberta, Canada (6, 7) (available upon request from C.L.N.). Briefly, bottles containing 50 ml of Kosmotoga olearia medium (1) were inoculated with 2 ml of FWKO water that had been stored anoxically at room temperature (RT) for 4 years, incubated at 55°C for 5 days, and then stored for 5 weeks at RT. Dilution series and bottle plates were made as described by Dipippo et al. (1) and incubated at 55°C for 3 weeks. One white round colony confirmed to be a Kosmotoga bacterium by 16S rRNA PCR was selected for genome sequencing.

DNA was extracted from 50-ml cultures of K. arenicorallina S304 and Kosmotoga sp. DU53, according to the protocol described by Charbonnier and Forterre (8). The purity and quantity of the DNA were measured using NanoDrop and Qubit instruments (Thermo Fisher Scientific). Kosmotoga sp. DU53 DNA was sheared using the Ion Shear Plus kit, and a library was constructed using the Ion Plus fragment library kit and sequenced on an Ion Torrent PGM (all from Life Technologies) using a 316 D Chip and 500 flows. The K. arenicorallina S304 library was constructed using the Nextera XT kit and sequenced as one of 10 pooled barcode libraries on a MiSeq (all from Illumina) using 250 cycles generating 2 × 250-bp paired-end reads.

The genome of K. arenicorallina S304 was assembled de novo by CLC Genomics Workbench 7.0.4, using trimming settings, automatic word size, a bubble size corresponding to the average length of the input reads, a minimum contig length of 1,000 bp, and reads mapped back to the contigs. Four contigs containing parts of two nonidentical 16S genes were located and manually resolved using its published 16S gene sequences (accession numbers AB530678 and AB530679). This resulted in 40 contigs totaling 2,113,627 bp, with an N50 of 109,886 bp, a longest contig size of 350,318 bp, and a G+C content of 41.0%

De novo assembly of the Kosmotoga sp. DU53 genome was done using MIRA 3, with Ion Torrent settings (9) (http://sourceforge.net/projects/mira-assembler/), resulting in 97 contigs totaling 2,375,260 bp, with an N50 of 66,806 bp, a longest contig size of 221,738 bp, and a G+C content of 41.4%

Both draft genomes were annotated in the NCBI Prokaryotic Genome Automatic Annotation Pipeline (PGAAP [10]), which identified 2,038 genes and 1,980 coding sequences (CDSs) for K. arenicorallina S304 and 2,504 genes and 2,430 CDSs for Kosmotoga sp. DU53.

Nucleotide sequence accession numbers. Both whole-genome shotgun projects have been deposited at DDBJ/EMBL/GenBank under the accession numbers JFHK00000000 and JGCK00000000 for Kosmotoga arenicorallina S304 and Kosmotoga sp. DU53, respectively. The versions described in this paper are the first versions, JFHK01000000 and JGCK01000000.

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REFERENCES
1. Dipippo JL, Nesbo CL, Dahle H, Doolittle WF, Birklad N-K, Noll KM. 2009. Kosmotoga olearia gen. nov., sp. nov., a thermophilic, anaerobic
heterotroph isolated from an oil production fluid. Int J Syst Evol Microbiol 59:2991–3000. http://dx.doi.org/10.1099/ijs.0.008045-0.

2. Nunoura T, Hirai M, Imachi H, Miyazaki M, Makita H, Hirayama H, Furuhashi Y, Yamamoto H, Takai K. 2010. Kosmotoga arenicorallina sp. nov. a thermophilic and obligately anaerobic heterotroph isolated from a shallow hydrothermal system occurring within a coral reef, southern part of the Yaeyama archipelago, Japan, reclassification of Thermococcoides shengliensis as Kosmotoga shengliensis comb. nov., and emended description of the genus Kosmotoga. Arch Microbiol 192:811–819. http://dx.doi.org/10.1007/s00203-010-0611-7.

3. Swithers KS, Dipippo JL, Bruce DC, Detter C, Tapia R, Han S, Goodwin LA, Han J, Woyke T, Pitluck S, Pennacchio I, Nolan M, Mikhailova N, Land ML, Nesbø CL, Gogarten JP, Noll KM. 2011. Genome sequence of Kosmotoga olearia strain TBF 19.5.1, a thermophilic bacterium with a wide growth temperature range, isolated from the Troll B oil platform in the North Sea. J Bacteriol 193:5566–5567. http://dx.doi.org/10.1128/JB.05828-11.

4. Nesbø CL, Bradman DM, Adebusuyi A, Dlutek M, Petrus AK, Fught J, Doolittle WF, Noll KM. 2012. Mesotoga prima gen. nov., sp. nov., the first described mesophilic species of the Thermotogales. Extremophiles 16:387–393. http://dx.doi.org/10.1007/s00792-012-0437-0.

5. Pollo SMJ, Zhaxybayeva O, Nesbø CL. 2015. Insights into thermoadaptation and the evolution of mesophily from the bacterial phylum Thermo- togae. Can J Microbiol 61:655–670. http://dx.doi.org/10.1139/cjm-2015-0073.

6. Nesbø CL, Kumaraswamy R, Dlutek M, Doolittle WF, Fught J. 2010. Searching for mesophilic Thermotogales bacteria: “mesotogas” in the wild. Appl Environ Microbiol 76:4896–4900. http://dx.doi.org/10.1128/AEM.02846-09.

7. Hulecki JC, Fught JM, Fedorak PM. 2010. Storage of oil field-produced waters alters their chemical and microbiological characteristics. J Ind Microbiol Biotechnol 37:471–481. http://dx.doi.org/10.1007/s10295-010-0693-x.

8. Charbonnier F, Forterre P. 1994. Comparison of plasmid DNA topology among mesophilic and thermophilic eubacteria and archaeabacteria. J Bacteriol 176:1251–1259.

9. Chevreux B, Wetter T, Suhai S. 1999. Genome sequence assembly using trace signals and additional sequence information, p 45–56. In Computer science and biology. Proceedings of the German Conference on Bioinformatics, GCB ’99. GCB, Hannover, Germany.

10. Angiuoli SV, Gussman A, Klimke W, Cochrane G, Field D, Garrity G, Kodira CD, Kyrpides N, Madupu R, Markowitz V, Tatusova T, Thomson N, White O. 2008. Toward an online repository of Standard Operating Procedures (SOPs) for (Meta)genomic annotation. Omics 12:137–141. http://dx.doi.org/10.1089/omi.2008.0017.