



Genome Sequence of an *Alkaliphilus* Species Isolated from Historically Contaminated Sediments of the Gulf of Naples (Mediterranean Sea)

Filippo Dell'Anno,^a Leonardo Joaquim van Zyl,^b Marla Trindade,^b Christophe Brunet,^a Antonio Dell'Anno,^c Adrianna Ianora,^a Clementina Sansone^a

^aStazione Zoologica Anton Dohrn, Istituto Nazionale di Biologia, Ecologia, e Biotecnologie Marine, Naples, Italy

^bInstitute for Microbial Biotechnology and Metagenomics, Department of Biotechnology, University of the Western Cape, Cape Town, South Africa

^cDepartment of Life and Environmental Science, Università Politecnica delle Marche, Ancona, Italy

ABSTRACT Here, we report the draft genome sequence of a metagenome-assembled genome (MAG) of a new *Alkaliphilus* bacterium, NP8, of the *Clostridiaceae* family. This bacterium was isolated from polluted sediment collected from an abandoned industrial site located in the Gulf of Naples (Mediterranean Sea) as part of a microbial consortium.

The ability of *Alkaliphilus* species to deal with harsh environmental conditions was described previously (1), e.g., an *Alkaliphilus transvaalensis* strain isolated from an ultradeep (3.2 km below the surface) gold mine. The metal-reducing capability reported for *Alkaliphilus* members (2, 3) likely plays a pivotal role in survival under extreme conditions. We report the metagenome-assembled genome (MAG) of a novel *Alkaliphilus* bacterium cultured as part of a microbial consortium from homogenized superficial marine sediments (0 to 20 cm) sampled through a Van Veen grab sampler in the Gulf of Naples (40°48'29.0"N, 14°09'54.7"E), which is highly contaminated by heavy metals and hydrocarbons (4). For sequencing, DNA was extracted from an enriched mixed microbial culture from marine sediment with the DNeasy blood and tissue kit (Qiagen) according to the manufacturer's instructions. Sequencing library preparation was performed using the Nextera DNA Flex kit (Illumina, Hayward, CA, USA) with 1 ng input DNA according to the manufacturer's instructions. The resulting libraries were sequenced on an Illumina MiSeq platform at the University of the Western Cape (Cape Town, South Africa) sequencing facility using a MiSeq reagent kit v2 (500 cycles) with a 10% phiX v3 spike generating 2 × 250-bp reads. Metagenome assembly was performed using CLC Genomics Workbench v7.5.1. The raw reads were trimmed and demultiplexed, and ≤500-bp contigs were removed from the final assembly. Binning of metagenomic contigs was performed using MyCC (<https://sourceforge.net/projects/sb2nhri/files/MyCC>) (5), while the completeness and contamination of the MAG, as well as genome quality, were determined with CheckM v1.0.18 using the lineage-specific workflow and default parameters (6). Gene prediction and annotation were performed using the Rapid Annotation using Subsystem Technology (RAST) pipeline (<http://rast.nmpdr.org>) (7) and the MicroScope pipeline (<https://mage.genoscope.cns.fr/microscope/home/index.php>) (8). The draft genome of *Alkaliphilus* NP8 is composed of 81 contigs totaling 2,673,585 bp, with a GC content of 29.1%, containing 2,661 putative genes with an average length of 822 bp (Fig. 1). CheckM analysis showed completeness of 98.6% and contamination of 0.6% (Table 1).

The organism belongs to the placeholder genus *Alkaliphilus* B. Taxonomy was assigned through whole-genome assessment against the Genome Taxonomy Database

Citation Dell'Anno F, van Zyl LJ, Trindade M, Brunet C, Dell'Anno A, Ianora A, Sansone C. 2021. Genome sequence of an *Alkaliphilus* species isolated from historically contaminated sediments of the Gulf of Naples (Mediterranean Sea). *Microbiol Resour Announc* 10:e00060-21. <https://doi.org/10.1128/MRA.00060-21>.

Editor Frank J. Stewart, Georgia Institute of Technology

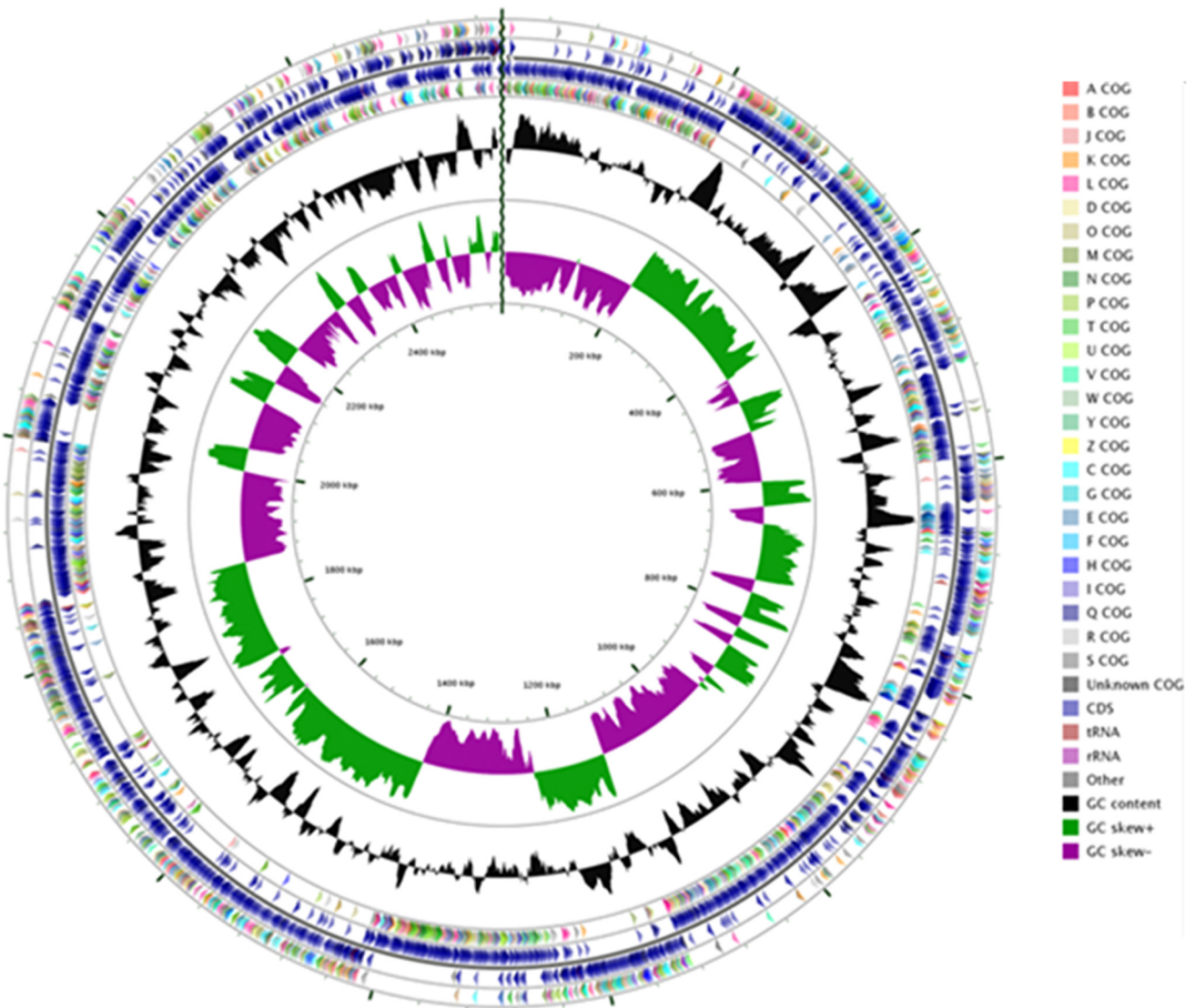
Copyright © 2021 Dell'Anno et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Filippo Dell'Anno, filippo.dellanno@szn.it.

Received 21 January 2021

Accepted 26 February 2021

Published 18 March 2021



Alkaliphilus NP 8

FIG 1 Circular representation of the *Alkaliphilus* bacterium NP8 genome using CGView Server^{Beta} (<http://cgview.ca>). The different rings represent (from outer to inner) predicted protein-coding sequences (CDS) on the forward (outer wheel) and reverse (inner wheel) strands (rings 2 and 3) colored according to the assigned Cluster of Orthologous Groups (COG) classes (rings 1 and 4), GC content (ring 5), GC skew (ring 6), and genomic position (ring 7). The key indicates the COG colors for the functional groups (A, RNA processing and modification; B, chromatin structure and dynamics; J, translation, ribosomal structure, and biogenesis; K, transcription; L, replication, recombination, and repair; D, cell cycle control, cell division, and chromosome partitioning; O, posttranslational modification, protein turnover, and chaperones; M, cell wall/membrane/envelope biogenesis; N, cell motility; P, inorganic ion transport and metabolism; T, signal transduction mechanisms; U, intracellular trafficking, secretion, and vesicular transport; V, defense mechanisms; W, extracellular structures; Y, nuclear structure; Z, cytoskeleton; C, energy production and conversion; G, carbohydrate transport and metabolism; E, amino acid transport and metabolism; F, nucleotide transport and metabolism; H, coenzyme transport and metabolism; I, lipid transport and metabolism; Q, secondary metabolite biosynthesis, transport, and catabolism; R, general function prediction only; S, function unknown).

v1.1.0 (<https://gtdb.ecogenomic.org>) as provided in KBase (https://kbase.us/applist/apps/kb_gtdbtk/run_kb_gtdbtk/release?gclid=CjwKCAiAyc2BBhAaEiwA44-wW7HAIOI9W WahrkAV0qleUu92NZCGEu34cla4XVbd3Vh0-xGBBH35NRoCOEUQAvD_BwE). To further explore the ability of *Alkaliphilus* NP8 to survive in polluted marine environments, we analyzed the gene functional categories provided by the annotation systems. The effective functionality of the observed genes has yet to be determined.

TABLE 1 General features of the genome of *Alkaliphilus* NP8

Parameter	Finding
CheckM results	
Completeness (%)	98.6
Contamination (%)	0.6
Size (bp)	2,673,585
GC content (%)	29.1
N_{50} (bp)	65,217
L_{50}	12
No. of contigs (with protein-encoding genes)	81
No. of subsystems	336
No. of coding sequences	2,671
No. with function assigned	1,827
No. hypothetical	844
No. of RNAs	36

In detail, two genes are involved in resistance to fluoroquinolones, while 18 genes are related to heavy metal detoxification, such as copper homeostasis and tolerance, cobalt, zinc, and cadmium resistance, mercuric reductase, the mercuric resistance operon, and multidrug resistance efflux pumps. We report 22 genes coding for superoxide dismutase and glutathione-related pathways, whose antioxidant and detoxification functions have already been described (9). The presence of genes involved in benzoate degradation, chloroalkane and chloroalkene degradation, naphthalene degradation, aminobenzoate degradation, and quinate degradation suggests the ability of *Alkaliphilus* NP8 to deal with hydrocarbon contamination.

Data availability. The draft genome sequence of *Alkaliphilus* NP8 was deposited under accession number [JADWMM0000000001](https://doi.org/10.1093/mra/mraa0000001) and BioProject number [PRJNA669418](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA669418). Whole-genome sequencing and Sequence Read Archive (SRA) records are available under accession numbers [JADWMM01](https://www.ncbi.nlm.nih.gov/sra/JADWMM01) and [SRR13496755](https://www.ncbi.nlm.nih.gov/sra/SRR13496755), respectively.

ACKNOWLEDGMENTS

F.D. was funded by a Ph.D. grant from the SZN and UNIVPM. This study was supported by the projects ABaCo (funded by the Italian Ministry for Education, University, and Research; grant C62F16000170001), Ocean Medicines (program H2020-MSCA-RISE-2015), and MERCES (program H2020-SC5-2015; grant 689518).

REFERENCES

- Kobayashi T, Lu J, Li Z, Hung VS, Kurata A, Hatada Y, Takai K, Ito S, Horikoshi K. 2007. Extremely high alkaline protease from a deep-subsurface bacterium, *Alkaliphilus transvaalensis*. *Appl Microbiol Biotechnol* 75:71–80. <https://doi.org/10.1007/s00253-006-0800-0>.
- Roh Y, Chon CM, Moon JW. 2007. Metal reduction and biomineralization by an alkaliphilic metal-reducing bacterium, *Alkaliphilus metalliredigens* (QYMF). *Geosci J* 11:415–423. <https://doi.org/10.1007/BF02857056>.
- Hwang C, Copeland A, Lucas S, Lapidus A, Barry K, Detter JC, Glavina del Rio T, Hammon N, Israni S, Dalin E, Tice H, Pitluck S, Chertkov O, Brettin T, Bruce D, Han C, Schmutz J, Larimer F, Land ML, Hauser L, Kyrpides N, Mikhailova N, Ye Q, Zhou J, Richardson P, Fields MW. 2016. Complete genome sequence of *Alkaliphilus metalliredigens* strain QYMF, an alkaliphilic and metal-reducing bacterium isolated from borax-contaminated leachate ponds. *Genome Announc* 4:3–4. <https://doi.org/10.1128/genomeA.01226-16>.
- Romano E, Bergamin L, Ausili A, Pierfranceschi G, Maggi C, Sesta G, Gabellini M. 2009. The impact of the Bagnoli industrial site (Naples, Italy) on sea-bottom environment: chemical and textural features of sediments and the related response of benthic foraminifera. *Mar Pollut Bull* 59:245–256. <https://doi.org/10.1016/j.marpolbul.2009.09.017>.
- Lin HH, Liao YC. 2016. Accurate binning of metagenomic contigs via automated clustering sequences using information of genomic signatures and marker genes. *Sci Rep* 6:12–19. <https://doi.org/10.1038/srep24175>.
- Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res* 25:1043–1055. <https://doi.org/10.1101/gr.186072.114>.
- Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F, Stevens R. 2014. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). *Nucleic Acids Res* 42:D206–D214. <https://doi.org/10.1093/nar/gkt1226>.
- Vallenet D, Calteau A, Cruveiller S, Gachet M, Lajus A, Josso A, Mercier J, Renaux A, Rollin J, Rouy Z, Roche D, Scarpelli C, Médigue C. 2017. MicroScope in 2017: an expanding and evolving integrated resource for community expertise of microbial genomes. *Nucleic Acids Res* 45:D517–D528. <https://doi.org/10.1093/nar/gkw1101>.
- Espinosa-Diez C, Miguel V, Mennerich D, Kietzmann T, Sánchez-Pérez P, Cadenas S, Lamas S. 2015. Antioxidant responses and cellular adjustments to oxidative stress. *Redox Biol* 6:183–197. <https://doi.org/10.1016/j.redox.2015.07.008>.