

Genome Sequences of *Cupriavidus metallidurans* Strains NA1, NA4, and NE12, Isolated from Space Equipment

Pieter Monsieurs,^a Kristel Mijndonckx,^a Ann Provoost,^a Kasthuri Venkateswaran,^b C. Mark Ott,^c Natalie Leys,^a Rob Van Houdt^a

Unit of Microbiology, Belgian Nuclear Research Centre (SCK•CEN), Mol, Belgium^a; Biotechnology and Planetary Protection Group, Jet Propulsion Laboratory, California Institute of Technology, Pasadena, California, USA^b; Biomedical Research and Environmental Science Division, NASA, Johnson Space Center, Houston, Texas, USA^c

***Cupriavidus metallidurans* NA1, NA4, and NE12 were isolated from space and spacecraft-associated environments. Here, we report their draft genome sequences with the aim of gaining insight into their potential to adapt to these environments.**

Received 24 June 2014 Accepted 3 July 2014 Published 24 July 2014

Citation Monsieurs P, Mijndonckx K, Provoost A, Venkateswaran K, Ott CM, Leys N, Van Houdt R. 2014. Genome sequences of *Cupriavidus metallidurans* strains NA1, NA4, and NE12, isolated from space equipment. *Genome Announc.* 2(4):e00719-14. doi:10.1128/genomeA.00719-14.

Copyright © 2014 Monsieurs 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 Rob Van Houdt, rob.van.houdt@sckcen.be.

Cupriavidus metallidurans strains have previously been isolated from industrial sites linked to mining, metallurgic, and chemical industries (1–3) and are characterized by multiple metal resistances (1, 4–8). A substantial portion of the metal-resistance determinants are carried by native megaplasmids such as pMOL28 and pMOL30 of the type strain CH34 (6, 9, 10). In addition, some *C. metallidurans* strains carry an extensive mobile gene pool (5, 11, 12). *C. metallidurans* NA1, NA4, and NE12 were isolated from water samples of the potable water system and the condensate water regeneration system of the International Space Station and from air particulates of the Payload Hazardous Servicing Facility located at the Kennedy Space Center in Florida, respectively (4, 13, 14). These isolates were sequenced for comparative analyses and for exploring the adaptation potential to these oligotrophic environments.

Whole-genome shotgun sequencing and paired-end sequencing of *C. metallidurans* NA4 and NE12 were performed by Macrogen (Seoul, South Korea) using the 454 GS-FLX sequencing platform. The sequencing data for strain NA4 and strain NE12 showed average read lengths of 307 nucleotides (nt) and 333 nt, average insert sizes of 2,972 nt and 2,777 nt, and total sequencing data of 258 Mbp and 409 Mbp, respectively. Both genomes were assembled using Newbler software (version 2.3), resulting in 109 and 87 contigs and N_{50} values of 226,033 nt and 197,312 nt for NA4 and NE12, respectively. Full-genome sequencing of *C. metallidurans* NA1 was performed by Baseclear (Leiden, The Netherlands) on the Illumina HiSeq 2000 platform using a combination of a paired-end sequencing library (101 nucleotides) with an average insert size of 240 nt and a mate-pair sequencing library (51 nucleotides) with an average insert size of 3,233 nt. Reads were filtered to remove low-quality reads, resulting in a total of 5,832,127 paired reads (594 Mbp) and 9,473,070 mate-pair reads (1,913 Mbp). *De novo* genome assembly based on the paired-end reads was performed using Velvet (15), resulting in 216 contigs and an N_{50} value of 69,748 nt. Applying the scaffolding algorithm SSPACE (16) using the mate-pair library resulted in 27 scaffolds.

The genome of *C. metallidurans* NA1 was estimated to be 6,833,318 bp, with a G+C content of 63.76%. The genome of NA4

was estimated to be 7,370,364 bp, with a G+C content of 63.27%. The genome of NE12 was estimated to be 7,132,975 bp, with a G+C content of 63.64%.

Next to the chromosome and the chromid (17), all three strains carry a megaplasmid comparable in size to pMOL30 (~234 kb). Strains NA1 and NA4 carry a second megaplasmid comparable in size to pMOL28 (~172 kb) (4). Strain NA4 carries an additional plasmid of around 95 kb (4).

The NA1 genome annotation through the MicroScope platform (18) displayed 6,815 coding sequences (CDS), of which 75.6% were classified in at least one cluster of orthologous groups (COG). NA4 displayed 7,467 CDS, of which 70.9% were classified in at least one COG. NE12 displayed 7,026 CDS, of which 74.3% were classified in at least one COG.

Nucleotide sequence accession numbers. These whole-genome shotgun projects have been deposited at DDBJ/EMBL/GenBank under the accession numbers [JFZD000000000](https://www.ncbi.nlm.nih.gov/nuccore/JFZD000000000) (version JFZD01000000), [JFZE000000000](https://www.ncbi.nlm.nih.gov/nuccore/JFZE000000000) (version JFZE01000000), and [JFZF000000000](https://www.ncbi.nlm.nih.gov/nuccore/JFZF000000000) (version JFZF01000000) for strains NA1, NA4 and NE12, respectively.

ACKNOWLEDGMENTS

This work was supported by the European Space Agency (ESA-PRODEX) and the Belgian Science Policy (Belspo) through the COMICS project (C90356).

REFERENCES

1. Goris J, De Vos P, Coenye T, Hoste B, Janssens D, Brim H, Diels L, Mergeay M, Kersters K, Vandamme P. 2001. Classification of metal-resistant bacteria from industrial biotopes as *Ralstonia campinensis* sp. nov., *Ralstonia metallidurans* sp. nov. and *Ralstonia basilensis* Steinle et al. 1998 emend. *Int. J. Syst. Evol. Microbiol.* 51:1773–1782. <http://dx.doi.org/10.1099/00207713-51-5-1773>.
2. Brim H, Heyndrickx M, de Vos P, Wilmotte A, Springael D, Schlegel HG, Mergeay M. 1999. Amplified rDNA restriction analysis and further genotypic characterisation of metal-resistant soil bacteria and related facultative hydrogenotrophs. *Syst. Appl. Microbiol.* 22:258–268. [http://dx.doi.org/10.1016/S0723-2020\(99\)80073-3](http://dx.doi.org/10.1016/S0723-2020(99)80073-3).
3. Diels L, Mergeay M. 1990. DNA probe-mediated detection of resistant bacteria from soils highly polluted by heavy metals. *Appl. Environ. Microbiol.* 56:1485–1491.

4. Mijnendonckx K, Provoost A, Ott CM, Venkateswaran K, Mahillon J, Leys N, Van Houdt R. 2013. Characterization of the survival ability of *Cupriavidus metallidurans* and *Ralstonia pickettii* from space-related environments. *Microb. Ecol.* 65:347–360. <http://dx.doi.org/10.1007/s00248-012-0139-2>.
5. Van Houdt R, Monsieurs P, Mijnendonckx K, Provoost A, Janssen A, Mergeay M, Leys N. 2012. Variation in genomic islands contributes to genome plasticity in *Cupriavidus metallidurans*. *BMC Genomics* 13:111. <http://dx.doi.org/10.1186/1471-2164-13-111>.
6. Janssen PJ, Van Houdt R, Moors H, Monsieurs P, Morin N, Michaux A, Benotmane MA, Leys N, Vallaëys T, Lapidus A, Monchy S, Médigue C, Taghavi S, McCorkle S, Dunn J, van der Lelie D, Mergeay M. 2010. The complete genome sequence of *Cupriavidus metallidurans* strain CH34, a master survivalist in harsh and anthropogenic environments. *PLoS One* 5:e10433. <http://dx.doi.org/10.1371/journal.pone.0010433>.
7. Mergeay M. 2000. Bacteria adapted to industrial biotopes: the metal resistant *Ralstonia*, p 403–414. In Storz G, Hengge-Aronis R (ed), *Bacterial stress responses*. ASM Press, Washington, DC.
8. Mergeay M, Monchy S, Vallaëys T, Auquier V, Benotmane A, Bertin P, Taghavi S, Dunn J, van der Lelie D, Wattiez R. 2003. *Ralstonia metallidurans*, a bacterium specifically adapted to toxic metals: towards a catalogue of metal-responsive genes. *FEMS Microbiol. Rev.* 27:385–410. [http://dx.doi.org/10.1016/S0168-6445\(03\)00045-7](http://dx.doi.org/10.1016/S0168-6445(03)00045-7).
9. Mergeay M, Monchy S, Janssen P, Van Houdt R, Leys N. 2009. Megaplasmids in *Cupriavidus* genus and metal resistance, p 209–238. In Schwartz E (ed), *Microbial megaplasmids*, vol 11. Springer Verlag, Berlin, Germany.
10. Monchy S, Benotmane MA, Janssen P, Vallaëys T, Taghavi S, van der Lelie D, Mergeay M. 2007. Plasmids pMOL28 and pMOL30 of *Cupriavidus metallidurans* are specialized in the maximal viable response to heavy metals. *J. Bacteriol.* 189:7417–7425. <http://dx.doi.org/10.1128/JB.00375-07>.
11. Van Houdt R, Monchy S, Leys N, Mergeay M. 2009. New mobile genetic elements in *Cupriavidus metallidurans* CH34, their possible roles and occurrence in other bacteria. *Antonie van Leeuwenhoek* 96:205–226. <http://dx.doi.org/10.1007/s10482-009-9345-4>.
12. Mijnendonckx K, Provoost A, Monsieurs P, Leys N, Mergeay M, Mahillon J, Van Houdt R. 2011. Insertion sequence elements in *Cupriavidus metallidurans* CH34: distribution and role in adaptation. *Plasmid* 65:193–203. <http://dx.doi.org/10.1016/j.plasmid.2010.12.006>.
13. Newcombe DA, La Duc MT, Vaishampayan P, Venkateswaran K. 2008. Impact of assembly, testing and launch operations on the airborne bacterial diversity within a spacecraft assembly facility clean-room. *Int. J. Astrobiol.* 7:223–236. <http://dx.doi.org/10.1017/S1473550408004254>.
14. Bobe L, Kochetkov A, Soloukhin V, Andreichuk PO, Protasov NN, Sinyak Y. 2008. srv-k status aboard the International Space Station during Missions 15 and 16. SAE technical paper 2008-01-2191. <http://dx.doi.org/10.4271/2008-01-2191>.
15. 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>.
16. Boetzer M, Henkel CV, Jansen HJ, Butler D, Pirovano W. 2011. Scaffolding preassembled contigs using SSPACE. *Bioinformatics* 27: 578–579. <http://dx.doi.org/10.1093/bioinformatics/btq683>.
17. Van Houdt R, Mergeay M. 2012. Plasmids as secondary chromosomes. In Bell E, Bond J, Klinman J, Masters B, Wells R (ed), *Molecular life sciences: an encyclopedic reference*: SpringerReference. Springer-Verlag, Heidelberg, Germany.
18. Vallenet D, Belda E, Calteau A, Cruveiller S, Engelen S, Lajus A, Le Fèvre F, Longin C, Mornico D, Roche D, Rouy Z, Salvignol G, Scarpelli C, Thil Smith AA, Weiman M, Médigue C. 2013. Microscope—an integrated microbial resource for the curation and comparative analysis of genomic and metabolic data. *Nucleic Acids Res.* 41:D636–D647. <http://dx.doi.org/10.1093/nar/gks1194>.