



## Draft Genome Sequence of *Enterobacter oligotrophicus*, Isolated from the Microbiome of a Lizard in the Caribbean

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**ABSTRACT** Here, we describe the genome sequence of ECC486. This *Enterobacter oligotrophicus* strain was isolated from a wild specimen of *Anolis marmoratus speciosus*, a lizard endemic to the territory of Guadeloupe (French West Indies). Its draft genome sequence consists of 40 contigs and contains a total of 4,504,233 bp, with a G+C content of 54.1%.

*E* nterobacter cloacae complex (ECC) members are isolated from various environments and recognized to be opportunistic pathogens (1). They harbor a chromosomic *ampC* cephalosporinase gene and are capable of exchanging resistance plasmids (1, 2). The introduction of genome sequencing and computational analysis revealed high genomic diversity in this complex, and its classification has been revised several times (3–6).

The present strain was isolated from a wild *Anolis marmoratus speciosus* specimen in Guadeloupe in October 2018. The lizard was caught in an urban area (16.228871 N, 61.521655 W) (7). A fresh fecal sample was recovered according to the approved procedure and inoculated overnight at 37°C into chromogenic agar (CCA; CHROMagar, Paris, France), after a pre-enrichment step on buffered peptone water (7). A single strain was isolated and initially identified as *Enterobacter hormaechei* (99.9%) by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) and the associated software (VITEK MS; bioMérieux, Marcy L'Etoile, France) (8).

A pure isolate was obtained after inoculation into fresh CCA medium and cultivated aerobically overnight at 37°C. The genomic DNA was extracted using the QIAamp DNA minikit (Qiagen, Hilden, Germany). After a quality check, a typing experiment was performed by amplifying a partial gene coding for heat shock protein 60 (hsp60) (5, 7). The amplicon was sequenced at Eurofins (286 bp; Eurofins Genomic SAS, Les Ullis, France). BLASTN submission on GenBank version 2.11.0 (https://blast.ncbi.nlm.nih.gov/Blast.cgi) indicated 100% coverage and a maximum percent nucleotide identity with the *Enterobacter oligotrophicus* reference strain CCA6 (99.30%; GenBank accession number AP019007.1) (9, 10).

The whole genome of strain ECC486 was sequenced to confirm typing observations (see "UD5" in Fig. S1 in the supplementary material for reference 7). This step was performed using a NextSeq 500 system (Illumina; Nextera XT kit library; 150-bp paired-end configuration). Unless otherwise indicated, default parameters were used for all the following software tools. The sequencing step generated a total of 7,081,784 raw reads, which were trimmed and filtered using AlienTrimmer version 0.4.0 (11). *De novo* assembly and annotation were performed using SPAdes version 3.12.0 ("–careful" option) and the Prokaryotic Genome Annotation Pipeline (PGAP) version 5.2 (12, 13). We obtained a 4,504,233-bp long genome sequence assembled into 40 contigs (G+C content, 54.1%). It has an  $N_{50}$  value of 257,079 bp and a single-copy BUSCO score of 99.5% completeness, for a 94-fold coverage (BUSCO version 5.0.0; QUAST version 5.0.2) (14, 15).

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Received 24 June 2021 Accepted 8 August 2021 Published 2 September 2021 The average nucleotide identity (ANI) was calculated using OrthoANI software version 0.93.1 against a reference panel (3, 16). In addition, determination of closely related validated reference genomes was performed using the later version of the TYGS platform and digital DNA:DNA hybridization (dDDH) (17). The results indicated that ECC486 is closely related to the *E. oligotrophicus* reference strain with an ANI value of 99.12% and a dDDH value of 93.5% (formula  $d_4$ ) (3, 17, 18). It underlined the usefulness of the hsp60 approach for an initial and less expensive *E. oligotrophicus* screening among this bacterial complex (5, 7, 9). To date, only two associated complete genome sequences are available. These strains were

(CCA6; AP019007.1) from leaf soil samples in Japan (3, 9, 19). As this bacterium was isolated from a lizard known to live near households, we determined the probability of its being a human pathogen in a "one health" perspective using PathogenFinder version 1.1 (82.2%) (20). Furthermore, in accordance to its previous antimicrobial susceptibility profile, resistance and plasmid analyses only identified a *bla*<sub>ACT</sub>-like gene (selected percent nucleotide identity threshold, 80%; minimum length, 80% for ResFinder version 4.1 and PlasmidFinder version 2.1) (7, 21, 22). Finally, the CRISPRCasFinder version 1.1.2 and PHASTER (upgrade 6) Web tools allowed us to clearly identify a CRISPR/Cas system (2 array sequences with an evidence level of 4; Cas type I-F) and an intact prophage sequence similar to that of strain HK225 (43.6 kb; GenBank accession number NC\_019717) (23, 24).

recovered in 1999 (CCA3; GenBank accession number NZ\_BNJN00000000.1) and 2016

**Data availability.** The NCBI BioProject accession number PRJNA730279 contains the ECC486 annotated genome sequence (GenBank accession number JAHCLV000000000) and the trimmed and filtered reads (SRA accession number SRR14559665). The initial partial sequence encoding hsp60 can be found under GenBank accession number MZ217779.

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## REFERENCES

- Davin-Regli A, Lavigne J-P, Pagès J-M. 2019. Enterobacter spp.: update on taxonomy, clinical aspects, and emerging antimicrobial resistance. Clin Microbiol Rev 32:e00002-19. https://doi.org/10.1128/CMR.00002-19.
- 2. Pot M, Guyomard-Rabenirina S, Couvin D, Ducat C, Enouf V, Ferdinand F, Gruel G, Malpote E, Talarmin A, Breurec S, Reynaud Y. 2021. Dissemination of extended-spectrum-β-lactamase-producing *Enterobacter cloacae* complex from a hospital to the nearby environment in Guadeloupe (French West Indies): ST114 lineage coding for a successful incHl2/ST1 plasmid. Antimicrob Agents Chemother 65:e02146-20. https://doi.org/10.1128/AAC.02146-20.
- Wu W, Feng Y, Zong Z. 2020. Precise species identification for Enterobacter: a genome sequence-based study with reporting of two novel species, Enterobacter quasiroggenkampii sp. nov. and Enterobacter quasimori sp. nov. mSystems 5:e00527-20. https://doi.org/10.1128/mSystems.00527-20.
- Sutton GG, Brinkac LM, Clarke TH, Fouts DE. 2018. Enterobacter hormaechei subsp. hoffmannii subsp. nov., Enterobacter hormaechei subsp. xiangfangensis comb. nov., Enterobacter roggenkampii sp. nov., and Enterobacter muelleri is a later heterotypic synonym of Enterobacter asburiae based on computational analysis of sequenced Enterobacter genomes. F1000Res 7:521. https://doi.org/10.12688/f1000research.14566.2.
- Hoffmann H, Roggenkamp A. 2003. Population genetics of the nomenspecies *Enterobacter cloacae*. Appl Environ Microbiol 69:5306–5318. https://doi.org/10.1128/AEM.69.9.5306-5318.2003.
- 6. Brady C, Cleenwerck I, Venter S, Coutinho T, De Vos P. 2013. Taxonomic evaluation of the genus *Enterobacter* based on multilocus sequence analysis (MLSA): proposal to reclassify *E. nimipressuralis* and *E. amnigenus* into *Lelliottia* gen. nov. as *Lelliottia nimipressuralis* comb. nov. and *Lelliottia amnigena* comb. nov., respectively, *E. gergoviae* and *E. pyrinus* into *Pluralibacter* gergoviae comb. nov. and *Pluralibacter* pyrinus comb. nov., respectively, *E. cowanii, E. radicincitans, E. oryzae* and *E. arachidis* into *Kosakonia* gen. nov. as *Kosakonia radicincitans* comb. nov., and

Kosakonia arachidis comb. nov., respectively, and E. turicensis, E. helveticus and E. pulveris into Cronobacter as Cronobacter zurichensis nom. nov., Cronobacter helveticus comb. nov. and Cronobacter pulveris comb. nov., respectively, and emended description of the genera Enterobacter and Cronobacter. Syst Appl Microbiol 36:309–319. https://doi.org/10.1016/j .syapm.2013.03.005.

- Pot M, Reynaud Y, Couvin D, Ducat C, Ferdinand S, Gravey F, Gruel G, Guérin F, Malpote E, Breurec S, Talarmin A, Guyomard-Rabenirina S. 2021. Wide distribution and specific resistance pattern to third-generation cephalosporins of *Enterobacter cloacae* complex members in humans and in the environment in Guadeloupe (French West Indies). Front Microbiol 12:628058. https://doi.org/10.3389/fmicb.2021.628058.
- Patel R. 2013. Matrix-assisted laser desorption ionization-time of flight mass spectrometry in clinical microbiology. Clin Infect Dis 57:564–572. https://doi.org/10.1093/cid/cit247.
- 9. Akita H, Matsushika A, Kimura Z-I. 2019. *Enterobacter oligotrophica* sp. nov., a novel oligotroph isolated from leaf soil. Microbiologyopen 8: e00843. https://doi.org/10.1002/mbo3.843.
- Oren A, Garrity GM. 2020. List of new names and new combinations previously effectively, but not validly, published. Int J Syst Evol Microbiol 70: 2960–2966. https://doi.org/10.1099/ijsem.0.004156.
- Criscuolo A, Brisse S. 2014. AlienTrimmer removes adapter oligonucleotides with high sensitivity in short-insert paired-end reads. Commentary on Turner (2014) assessment of insert sizes and adapter content in FASTQ data from NexteraXT libraries. Front Genet 5:130. https://doi.org/10.3389/ fgene.2014.00130.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.

- Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KM, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. Nucleic Acids Res 44:6614–6624. https://doi.org/ 10.1093/nar/gkw569.
- Simão FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM. 2015. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics 31:3210–3212. https://doi.org/ 10.1093/bioinformatics/btv351.
- Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29:1072–1075. https:// doi.org/10.1093/bioinformatics/btt086.
- Lee I, Kim YO, Park S-C, Chun J. 2016. OrthoANI: an improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microbiol 66:1100–1103. https://doi.org/10.1099/ijsem.0.000760.
- Meier-Kolthoff JP, Göker M. 2019. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nat Commun 10: 2182. https://doi.org/10.1038/s41467-019-10210-3.
- Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M. 2013. Genome sequencebased species delimitation with confidence intervals and improved distance functions. BMC Bioinformatics 14:60. https://doi.org/10.1186/1471 -2105-14-60.
- 19. Akita H, Itoiri Y, Takeda N, Kimura Z-I, Inoue H, Matsushika A. 2021. Draft genome sequence of *Enterobacter oligotrophicus* CCA3, isolated from leaf

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soil. Microbiol Resour Announc 10:e01289-20. https://doi.org/10.1128/ MRA.01289-20.

- Cosentino S, Larsen MV, Aarestrup FM, Lund O. 2013. Correction: PathogenFinder—distinguishing friend from foe using bacterial whole genome sequence data. PLoS One 8:1–11. https://doi.org/10.1371/annotation/ b84e1af7-c127-45c3-be22-76abd977600f.
- Carattoli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O, Villa L, Moller Aarestrup F, Hasman H. 2014. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother 58:3895–3903. https://doi.org/10.1128/ AAC.02412-14.
- Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV. 2012. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 67:2640–2644. https://doi.org/ 10.1093/jac/dks261.
- Couvin D, Bernheim A, Toffano-Nioche C, Touchon M, Michalik J, Néron B, Rocha EPC, Vergnaud G, Gautheret D, Pourcel C. 2018. CRISPRCasFinder, an update of CRISRFinder, includes a portable version, enhanced performance and integrates search for Cas proteins. Nucleic Acids Res 46: W246–W251. https://doi.org/10.1093/nar/gky425.
- Arndt D, Grant JR, Marcu A, Sajed T, Pon A, Liang Y, Wishart DS. 2016. PHASTER: a better, faster version of the PHAST phage search tool. Nucleic Acids Res 44:W16–W21. https://doi.org/10.1093/nar/gkw387.