



Complete Closed Genome Sequence of Nontoxigenic Invasive *Corynebacterium diphtheriae* bv. mitis Strain ISS 3319

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ABSTRACT The genome sequence of the human pathogen *Corynebacterium diphtheriae* bv. mitis strain ISS 3319 was determined and closed in this study. The genome is estimated to have 2,404,936 bp encoding 2,257 proteins. This strain also possesses a plasmid of 1,960 bp.

Corynebacterium diphtheriae is the etiological agent of diphtheria, a toxigenic infection of the upper respiratory tract. Besides diphtheria, systemic infections caused by nontoxigenic strains have been reported (1–5). The molecular basis of toxin-independent pathogenicity of *C. diphtheriae* is poorly understood (6). Therefore, we sequenced the whole genome of *C. diphtheriae* bv. mitis ISS 3319, isolated from the throat of 9-year-old patient (7, 8). The strain lacks the *tox* gene but is able to adhere to and invade human epithelial cells and effectively colonize spleen and kidneys of mice (9–11).

Sequencing of libraries was carried out with the Oxford Nanopore MinION instrument and the Illumina HiSeq platform using a 250-bp paired-end protocol. The Illumina and MinION reads were assembled together using Unicycler with Pilon polishing, with a final hybrid assembly of two contigs of 1,604,547 and 800,125 bp. Two gaps of 124 bp and 140 bp were identified in the genome using BLAST searches of >500 bp from the ends of both the contigs against the published assembly of the same strain (GenBank accession no. JAQN00000000). The contigs were merged using the missing sequence after aligning them to the genome sequence of strain C7 (beta) (GenBank accession no. NC_016801), which belongs to the same sequence type, ST26, as ISS 3319 (7). Automatic annotation and identification of rRNAs and tRNAs were accomplished using the Prokka version 1.11 software tool (12).

The new sequence was compared to the previously published assembly of ISS 3319, which was also reannotated using Prokka to obtain an equivalence of annotation for comparative analysis. The annotated genomes were compared using Roary (13, 14); 2,195 genes are conserved between the assemblies, and only four single nucleotide polymorphisms (SNPs) were observed in the core genomic alignment. Most of the 26 genes reported to be specific to the previous assembly (GenBank accession no. JAQN00000000) showed significant similarities to several genes in the new assembly and may not be specific. The proteins encoded by the majority of these genes are short and fall within or near the identified genomic islands that were identified using IslandViewer 4 (15).

The closed genome of 2,404,936 bp contained 9 rRNAs, 53 tRNAs, 1 transfer-messenger RNA, 1 repeat region, 2,257 protein-coding sequences, and 53.46% GC

Received 15 December 2017 **Accepted** 19 December 2017 **Published** 1 February 2018

Citation Azevedo Antunes C, Richardson EJ, Quick J, Fuentes-Utrilla P, Isom GL, Goodall EC, Möller J, Hoskisson PA, Mattos-Guaraldi AL, Cunningham AF, Loman NJ, Sangal V, Burkovski A, Henderson IR. 2018. Complete closed genome sequence of nontoxigenic invasive *Corynebacterium diphtheriae* bv. mitis strain ISS 3319. *Genome Announc* 6:e01566-17. <https://doi.org/10.1128/genomeA.01566-17>.

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content. Moreover, a circular plasmid of 1,960 bp with four coding sequences was also identified. A discontinuous mega-BLAST search of this plasmid sequence in GenBank revealed partial similarities (67% identity in 612-bp alignment) with plasmid pTA144 in *Moraxella* sp. (GenBank accession no. NC_001316) and the metamobilome of an uncultured strain (65% identity in 833-bp alignment; GenBank accession no. LN853682). A nonredundant translation alignment of the plasmid sequence showed a conserved domain with homology to the phage replication protein Cri, described as essential for DNA replication in *Vibrio cholerae* serotype O1 (16), and a sequence with 88% identity to a gene encoding an $\alpha\beta$ -hydrolase from a *Streptococcus* sp. (GenBank accession no. WP_002883881). Coinfections of *C. diphtheriae* with other pathogens, such as *Staphylococcus* spp., *Streptococcus* spp., *Arcanobacterium* spp., and *Pseudomonas* spp., were reported previously (8, 17, 18), and this close contact might support horizontal DNA transfer.

Accession number(s). The *C. diphtheriae* bv. mitis ISS 3319 whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. CP025209 and CP025210. The versions described in this paper are the second versions.

ACKNOWLEDGMENTS

Genome sequencing and bioinformatics were performed by MicrobesNG (<http://microbesng.uk>), which is supported by the BBSRC (grant BB/L024209/1). A.L.M.-G. and C.A.A. were supported by the Coordination for the Improvement of Higher Education Personnel (CAPES).

C.A.A. gratefully acknowledges the Förderung von Frauen in Forschung und Lehre (FFL) of the Friedrich-Alexander Universität Erlangen-Nürnberg.

REFERENCES

- Burkovski A. 2014. Diphtheria and its etiological agents, p 1–14. In Burkovski A (ed), *Corynebacterium diphtheriae* and related toxigenic species. Springer, Dordrecht, The Netherlands.
- Belchior E, Henry S, Badell E, Collet L, Benoit-Cattin T, de Montera AM, Guiso N, Patey O, Levy-Bruhl D, Filleul L, Chieze F, Olivier S. 2017. Diphtheria in Mayotte, 2007–2015. *Emerg Infect Dis* 23:1218–1220. <https://doi.org/10.3201/eid2307.170262>.
- Kolios AGA, Cozzio A, Zinkernagel AS, French LE, Kündig TM. 2017. Cutaneous *Corynebacterium* infection presenting with disseminated skin nodules and ulceration. *Case Rep Dermatol* 9:8–12. <https://doi.org/10.1159/000476054>.
- Santos LS, Sant'Anna LO, Ramos JN, Ladeira EM, Stavracakis-Peixoto R, Borges LL, Santos CS, Napoleão F, Camello TCF, Pereira GA, Hirata R, Vieira WV, Cosme LMSS, Sabbadini PS, Mattos-Guaraldi AL. 2015. Diphtheria outbreak in Maranhão, Brazil: microbiological, clinical and epidemiological aspects. *Epidemiol Infect* 143:791–798. <https://doi.org/10.1017/S0950268814001241>.
- Gomes DL, Martins CA, Faria LM, Santos LS, Santos CS, Sabbadini PS, Souza MC, Alves GB, Rosa AC, Nagao PE, Pereira GA, Hirata R, Mattos-Guaraldi AL. 2009. *Corynebacterium diphtheriae* as an emerging pathogen in nephrostomy catheter-related infection: evaluation of traits associated with bacterial virulence. *J Med Microbiol* 58:1419–1427. <https://doi.org/10.1099/jmm.0.012161-0>.
- Sangal V, Hoskisson PA. 2016. Evolution, epidemiology and diversity of *Corynebacterium diphtheriae*: new perspectives on an old foe. *Infect Genet Evol* 43:364–370. <https://doi.org/10.1016/j.meegid.2016.06.024>.
- Sangal V, Blom J, Sutcliffe IC, von Hunolstein C, Burkovski A, Hoskisson PA. 2015. Adherence and invasive properties of *Corynebacterium diphtheriae* strains correlates with the predicted membrane-associated and secreted proteome. *BMC Genomics* 16:765. <https://doi.org/10.1186/s12864-015-1980-8>.
- von Hunolstein C, Alfaroni G, Scopetti F, Pataracchia M, La Valle R, Franchi F, Pacciani L, Manera A, Giammanco A, Farinelli S, Engler K, De Zoysa A, Efstratiou A. 2003. Molecular epidemiology and characteristics of *Corynebacterium diphtheriae* and *Corynebacterium ulcerans* strains isolated in Italy during the 1990s. *J Med Microbiol* 52:181–188. <https://doi.org/10.1099/jmm.0.04864-0>.
- Ott L, Höller M, Gerlach RG, Hensel M, Rheinlaender J, Schäffer TE, Burkovski A. 2010. *Corynebacterium diphtheriae* invasion-associated protein (DIP1281) is involved in cell surface organization, adhesion and internalization in epithelial cells. *BMC Microbiol* 10:2. <https://doi.org/10.1186/1471-2180-10-2>.
- Ott L, Scholz B, Höller M, Hasselt K, Ensser A, Burkovski A. 2013. Induction of the NF- κ B signal transduction pathway in response to *Corynebacterium diphtheriae* infection. *Microbiology* 159:126–135. <https://doi.org/10.1099/mic.0.061879-0>.
- Puliti M, Von Hunolstein C, Marangi M, Bistoni F, Tissi L. 2006. Experimental model of infection with non-toxicigenic strains of *Corynebacterium diphtheriae* and development of septic arthritis. *J Med Microbiol* 55:229–235. <https://doi.org/10.1099/jmm.0.46135-0>.
- Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30:2068–2069. <https://doi.org/10.1093/bioinformatics/btu153>.
- Tange O. 2011. GNU parallel—the command-line power tool. *Linux Magazine* 36:42–47.
- Page AJ, Cummins CA, Hunt M, Wong VK, Reuter S, Holden MT, Fookes M, Falush D, Keane JA, Parkhill J. 2015. Roary: rapid large-scale prokaryote pan genome analysis. *Bioinformatics* 31:3691–3693. <https://doi.org/10.1093/bioinformatics/btv421>.
- Bertelli C, Laird MR, Williams KP, Simon Fraser University Research, Lau BY, Hoad G, Winsor GL, Brinkman FS. 2017. IslandViewer 4: expanded prediction of genomic islands for larger-scale datasets. *Nucleic Acids Res* 45:W30–W35. <https://doi.org/10.1093/nar/gkx343>.
- Heidelberg JF, Eisen JA, Nelson WC, Clayton RA, Gwinn ML, Dodson RJ, Haft DH, Hickey EK, Peterson JD, Umayam L, Gill SR, Nelson KE, Read TD, Tettelin H, Richardson D, Ermolaeva MD, Vamathevan J, Bass S, Qin H, Dragoi I, Sellers P, McDonald L, Utterback T, Fleishmann RD, Nierman WC, White O, Salzberg SL, Smith HO, Colwell RR, Mekalanos JJ, Venter JC, Fraser CM. 2000. DNA sequence of both chromosomes of the cholera pathogen *Vibrio cholerae*. *Nature* 406:477–483. <https://doi.org/10.1038/35020000>.
- Gordon CL, Fagan P, Hennessy J, Baird R. 2011. Characterization of *Corynebacterium diphtheriae* isolates from infected skin lesions in the Northern Territory of Australia. *J Clin Microbiol* 49:3960–3962. <https://doi.org/10.1128/JCM.05038-11>.
- May ML, McDougall RJ, Robson JM. 2014. *Corynebacterium diphtheriae* and the returned tropical traveler. *J Travel Med* 21:39–44. <https://doi.org/10.1111/jtm.12074>.