



## Complete Genome Sequence of the Oral Spirochete Bacterium *Treponema putidum* Strain OMZ 758<sup>T</sup> (ATCC 700334<sup>T</sup>)

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The oral spirochete bacterium *Treponema putidum* inhabits human periodontal niches. The complete genome sequence of the OMZ 758<sup>T</sup> (ATCC 700334<sup>T</sup>) strain of this species was determined, revealing a 2,796,913-bp chromosome, with a G+C content of 37.30% and a single plasmid (pTPu1; 3,649 bp) identical to pTS1 from *Treponema denticola*.

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ore than 75 species or phylotypes of oral spirochetes inhabit the oral cavity (1, 2). All taxa belong to the genus *Treponema* and are classified into 10 phylogroups (3). Phylogroup 2 contains two formally described species, *Treponema denticola* (4) and *Treponema putidum* (5), which share ca. 98.5% 16S rRNA gene sequence homology. The genome sequence of *T. denticola* ATCC 35405<sup>T</sup> was published in 2004 (6). A large number of clinical studies have associated *T. denticola* with human periodontal diseases (reviewed in references 7–9). Unlike *T. denticola*, the ecology, distributions, and clinical disease associations of *T. putidum* are poorly understood. Here, we report the complete genome of the type strain of *T. putidum* (OMZ 758<sup>T</sup>, ATCC 700334<sup>T</sup>), which was originally isolated from a Swiss periodontitis patient (5).

The OMZ  $758^{\rm T}$  strain was obtained directly from the original isolator and depositor (C. Wyss, University of Zurich) and was cultured anaerobically in supplemented tryptone-yeast extract-gelatin-volatile fatty acids-serum (TYGVS) medium, as previously described (10). Genomic DNA was extracted from a pure culture (QIAamp DNA mini extraction kit; Qiagen, Germany) and was sequenced using a 454 Life Sciences GS Junior platform at the Nanjing Agricultural University Bioinformatics Centre, Jiangsu, China. An initial shotgun library generated 245,625 reads, which was followed by an 8-kb span paired-end library yielding 119,479 reads, generating sequence data with  $54\times$  coverage. Using 454 Newbler version 2.7, the *de novo* assembly resulted in 40 large contigs, with an  $N_{50}$  contig size of 264,778 bp in two scaffolds. The single gap was closed by PCR and Sanger sequencing.

The *T. putidum* OMZ 758<sup>T</sup> chromosome is 2,796,913 bp, with a G+C content of 37.30%. Annotation was performed automatically using the PGAAP pipeline implemented in NCBI, following the best-placed reference protein set (GeneMarkS+ version 2.7). The genome contains 2,448 genes, 176 pseudogenes, 42 tRNAs, and two copies of an rRNA cluster (5S, 16S, and 23S). There is also a single plasmid of 3,649 bp (pTPu1), with a G+C content of 34.17%, which shares 100% sequence similarity with *T. denticola* plasmid pTS1 (accession no. AF112856) (11). The genome en-

codes homologues of virulence factors previously identified within *T. denticola* (7–9). These include the major surface protein (MSP) (locus tag JO40\_03880) involved in cellular adhesion processes (12), factor H binding protein (FhbB, FHL-1) (locus tag JO40\_03575), implicated in evading complement-mediated killing (13), and cystalysin (hemolysin [Hly]) (locus tag JO40\_09085), involved in volatile sulfur compound production and erythrolysis (14).

The annotated full-genome sequence for T. putidum OMZ  $758^{\rm T}$  (ATCC  $700334^{\rm T}$ ) reported here represents the first strain for this species. This significantly broadens the genomic data available for studying the biology, physiology, and phylogeny of oral treponemes associated with periodontal diseases. It will also facilitate efforts to systematically analyze genetically related species of treponemes that inhabit oral and nonoral niches in animals, many of which are implicated in polymicrobial tissue-destructive diseases (15, 16).

**Nucleotide sequence accession numbers.** This complete genome sequence has been deposited in GenBank under the accession no. CP009228. The pTPu1 plasmid sequence has been deposited under the accession no. CP009229.

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## REFERENCES

- Dewhirst FE, Chen T, Izard J, Paster BJ, Tanner AC, Yu WH, Lakshmanan A, Wade WG. 2010. The human oral microbiome. J. Bacteriol. 192:5002–5017. http://dx.doi.org/10.1128/JB.00542-10.
- 2. You M, Mo S, Leung WK, Watt RM. 2013. Comparative analysis of oral treponemes associated with periodontal health and disease. BMC Infect. Dis. 13:174. http://dx.doi.org/10.1186/1471-2334-13-174.

- Dewhirst FE, Tamer MA, Ericson RE, Lau CN, Levanos VA, Boches SK, Galvin JL, Paster BJ. 2000. The diversity of periodontal spirochetes by 16S rRNA analysis. Oral Microbiol. Immunol. 15:196–202. http://dx.doi.org/ 10.1034/j.1399-302x.2000.150308.x.
- 4. Chan EC, Siboo R, Keng T, Psarra N, Hurley R, Cheng SL, Iugovaz I. 1993. *Treponema denticola* (ex Brumpt 1925) sp. nov., nom. rev., and identification of new spirochete isolates from periodontal pockets. Int. J. Syst. Bacteriol. 43:196–203. http://dx.doi.org/10.1099/00207713-43-2-196.
- 5. Wyss C, Moter A, Choi BK, Dewhirst FE, Xue Y, Schüpbach P, Göbel UB, Paster BJ, Guggenheim B. 2004. *Treponema putidum* sp. nov., a medium-sized proteolytic spirochaete isolated from lesions of human periodontitis and acute necrotizing ulcerative gingivitis. Int. J. Syst. Evol. Microbiol. 54:1117–1122. http://dx.doi.org/10.1099/ijs.0.02806-0.
- 6. Seshadri R, Myers GS, Tettelin H, Eisen JA, Heidelberg JF, Dodson RJ, Davidsen TM, DeBoy RT, Fouts DE, Haft DH, Selengut J, Ren Q, Brinkac LM, Madupu R, Kolonay J, Durkin SA, Daugherty SC, Shetty J, Shvartsbeyn A, Gebregeorgis E, Geer K, Tsegaye G, Malek J, Ayodeji B, Shatsman S, McLeod MP, Smajs D, Howell JK, Pal S, Amin A, Vashisth P, McNeill TZ, Xiang Q, Sodergren E, Baca E, Weinstock GM, Norris SJ, Fraser CM, Paulsen IT. 2004. Comparison of the genome of the oral pathogen *Treponema denticola* with other spirochete genomes. Proc. Natl. Acad. Sci. U. S. A. 101:5646–5651. http://dx.doi.org/10.1073/pnas.0307639101.
- Ishihara K. 2010. Virulence factors of *Treponema denticola*. Periodontology 2000 54:117–135. http://dx.doi.org/10.1111/j.1600-0757.2009.00345.x.
- Dashper SG, Seers CA, Tan KH, Reynolds EC. 2011. Virulence factors of the oral spirochete *Treponema denticola*. J. Dent. Res. 90:691–703. http:// dx.doi.org/10.1177/0022034510385242.

- 9. Ellen RP, Galimanas VB. 2005. Spirochetes at the forefront of periodontal infections. Periodontology 2000 38:13–32. http://dx.doi.org/10.1111/j.1600-0757.2005.00108.x.
- Fenno JC. 2005. Laboratory maintenance of *Treponema denticola*. Curr. Protoc. Microbiol. Chapter 12:Unit 12.B.1. http://dx.doi.org/10.1002/9780471729259.mc12b01s00.
- 11. Chauhan S, Kuramitsu HK. 2004. Sequence analysis of plasmid pTS1 isolated from oral spirochetes. Plasmid 51:61–65. http://dx.doi.org/10.1016/j.plasmid.2003.11.002.
- 12. Fenno JC, Wong GW, Hannam PM, Müller KH, Leung WK, McBride BC. 1997. Conservation of *msp*, the gene encoding the major outer membrane protein of oral *Treponema* spp. J. Bacteriol. 179:1082–1089. http://jb.asm.org/content/179/4/1082.full.pdf.
- McDowell JV, Frederick J, Stamm L, Marconi RT. 2007. Identification of the gene encoding the FhbB protein of *Treponema denticola*, a highly unique factor H-like protein 1 binding protein. Infect. Immun. 75: 1050–1054. http://dx.doi.org/10.1128/IAI.01458-06.
- 14. Chu L, Burgum A, Kolodrubetz D, Holt SC. 1995. The 46-kilodalton-hemolysin gene from *Treponema denticola* encodes a novel hemolysin homologous to aminotransferases. Infect. Immun. 63:4448–4455. http://iai.asm.org/content/63/11/4448.long.
- Klitgaard K, Boye M, Capion N, Jensen TK. 2008. Evidence of multiple *Treponema* phylotypes involved in bovine digital dermatitis as shown by 16S rRNA gene analysis and fluorescence *in situ* hybridization. J. Clin. Microbiol. 46:3012–3020. http://dx.doi.org/10.1128/JCM.00670-08.
- Svartström O, Mushtaq M, Pringle M, Segerman B. 2013. Genome-wide relatedness of *Treponema pedis*, from gingiva and necrotic skin lesions of pigs, with the human oral pathogen *Treponema denticola*. PLoS One 8:e71281. http://dx.doi.org/10.1371/journal.pone.0071281.