

# Complete genome sequence of *Treponema denticola* KCOM 3500 isolated from the subgingival plaque of a human chronic periodontitis lesion

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## 사람 만성치주염 병소의 치은연하 치면세균막에서 분리한 *Treponema denticola* KCOM 3500의 전장 유전체 염기서열

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*Treponema denticola* is a strictly anaerobic, Gram-negative, non-spore forming and motile, spiral-shaped cells. It is a major causative agent of periodontitis and is related to systemic diseases such as Alzheimer's disease and cardiovascular diseases. *T. denticola* KCOM 3500 (= ChDC P019-Td1) was isolated from human chronic periodontitis lesion. In this study, we present the whole genome sequence of *T. denticola* KCOM 3500. The genome of strain KCOM 3500 was 2,778,422 bp in length and contained 2,507 protein-coding sequences, 6 rRNAs, and 44 tRNAs. The G + C content of it was 38.1%. It contained a dentilisin complex serine proteinase, a strong protease.

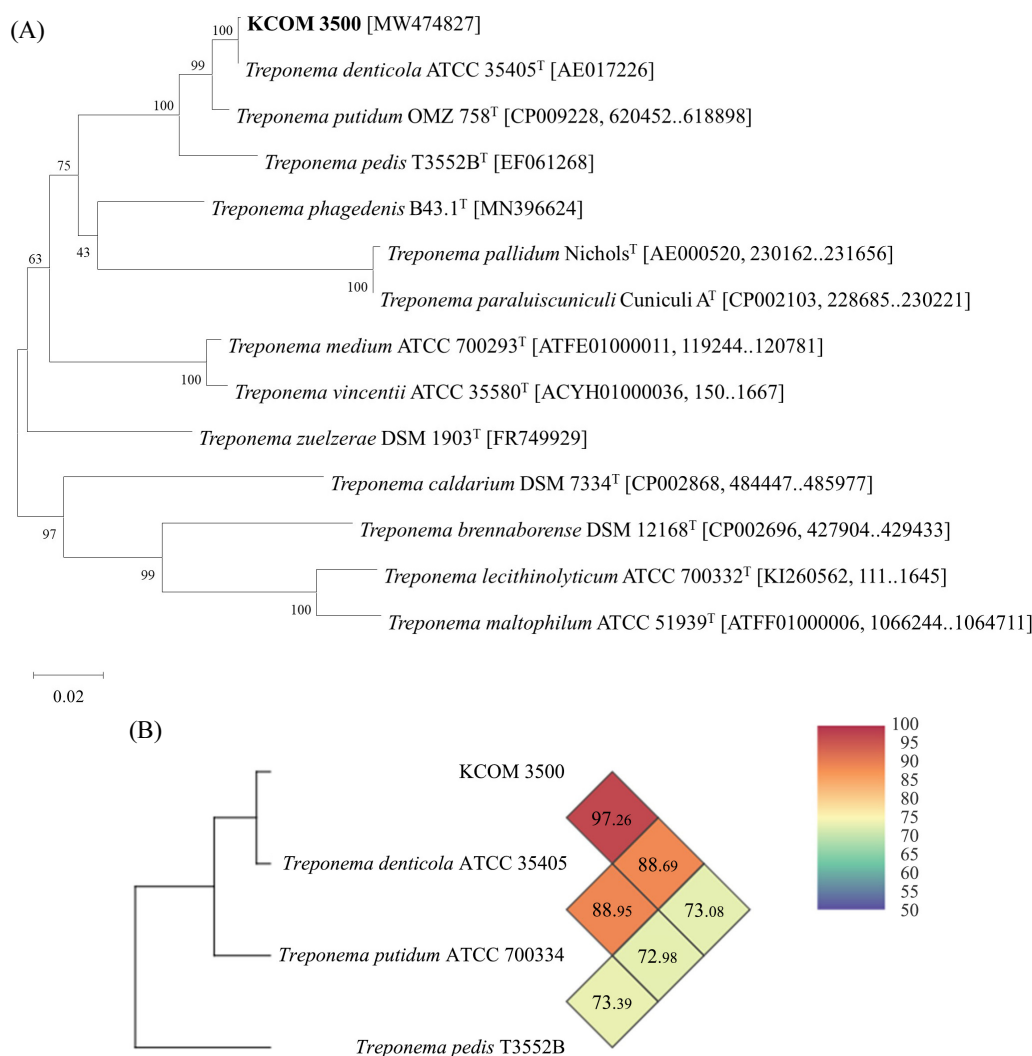
**Keywords:** *Treponema denticola*, chronic periodontitis, human

*Treponema denticola* is a Gram-stain-negative, strictly anaerobic, motile, and non-spore forming spiral-shaped cells (Chan *et al.*, 1993). *T. denticola* is frequently isolated from the subgingival dental plaque of chronic periodontitis lesions (Takeuchi *et al.*, 2001) and may also be found in periapical endodontic lesions (Gomes *et al.*, 2008). Recent epidemiological studies show that *T. denticola* is closely associated with

systemic diseases such as Alzheimer's disease (Su *et al.*, 2021) and cardiovascular diseases (Beck *et al.*, 2005). It has been reported that *T. denticola* has potential virulence genes, examples include: a major outer sheath membrane (Msp), an oligopeptide transporter unit (OppA), dentilisin (PrtP; a chymotrypsin-like protease), cystalysin (HlyA/HlyB: hemolyzes red blood cells), a hemin binding protein (HbpA/HbpB/troA-D/TroR), chemotaxis proteins (CheA/CheX/CheY/DmcA/DmcB), and FH-like binding protein (FhbB) (Ishihara, 2010).

Strain ChDC P019-Td1 was grown on agar plate using modified NOS medium (ATCC medium 1494; <https://www.atcc.org/products/35405>) from a subgingival dental plaque obtained from a male human suffering from chronic periodontitis (52 years old) in 2018 at the Chosun Dental Hospital (IRB No., CUDHIRB 1703001). The 16S rRNA gene (16S rDNA) was sequenced and deposited to GenBank (accession no. MW474827). To identify the strain at the species level, 16S rDNA sequence identification analysis was performed using a 16S-based ID program and the EzBioCloud database (<https://www.ezbiocloud.net>). The 16S rDNA sequence of the strain exhibited a 99.9% and 98.4% sequence identity with those of *T. denticola* ATCC 35405<sup>T</sup> (GenBank accession no. AE017226)

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**Fig. 1. (A)** Phylogenetic tree based on the 16S ribosomal RNA gene (16S rDNA) sequences showing the relationships between strain KCOM 3500 and type strains of closely-related *Treponema* species. It was constructed using neighbor-joining evolution methods in MEGA software (version 11, <https://megasoftware.net>). Stability of the trees was assessed using bootstrap analysis of 1000 replicates. The GenBank accession number and/or sequence region of the 16S rDNA of each type strain is written in parenthesis. Bar indicates 0.02 changes per nucleotide position. **(B)** Heatmap constructed based on whole genome sequences of strain KCOM 3500 and each type strain of the three *Treponema* sp. closest to *T. denticola* generated by OrthoANI calculator (Lee *et al.*, 2016). The GenBank accession numbers of *Treponema denticola* ATCC 35405<sup>T</sup>, *Treponema putidum* ATCC 700334<sup>T</sup>, and *Treponema pedis* T3552B<sup>T</sup> are NC\_002967, NZ\_VLLD01000001, and CP045670, respectively.

and *Treponema putidum* OMZ 758<sup>T</sup> (GenBank accession no. CP009228), respectively. The phylogenetic analysis based on 16S rDNA was performed using the Mega program (version 11, <https://megasoftware.net>). The phylogenetic tree data showed that strain KCOM 3500 was included in same cluster of *T. denticola* ATCC 35405<sup>T</sup> (Fig. 1A). The strain was identified as *T. denticola* and deposited in the Korean Collection for Oral Microbiology (KCOM) (deposit no.: KCOM 3500). In this report, we present the whole genome sequence of the strain, *T. denticola* KCOM 3500.

Strain KCOM 3500 was grown in a modified NOS medium in an anaerobic chamber (Model Bactron I) with a gaseous atmosphere of 85% N<sub>2</sub>, 10% CO<sub>2</sub>, and 5% H<sub>2</sub>. The bacterial genomic DNA was prepared as previously described (Cho *et al.*, 2015). The genomic DNA of strain KCOM 3500 was sequenced using a PacBio SequelI sequencing platform using a 20 kb SMRTbell template library by Macrogen Inc. Approximately 720,805,033 bases (259.4×) with 72,199 subreads (mean subreads length: 9,983 bases, N50 was 12,579 bases) were generated and assembled into a single contig by

**Table 1.** Genome features of the genome of *Treponema denticola* KCOM 3500

Attribute	Value
Genome size (bp)	2,778,422
GC content (%)	38.1
No. of contig	1
Total genes	2584
Protein-coding genes	2,507
tRNA	44
Complete rRNA (5S, 16S, 23S)	6 (2, 2, 2)
ncRNA	3
Pseudogene	24
CRISPR arrays	1

HGAP (version: 3.0, default setting) in PacBio's SMRT portal (<http://www.pacb.com/products-and-services/analytical-software/smrt-analysis>). Genome annotation was conducted using the NCBI Prokaryotic Genome Annotation Pipeline (Tatusova *et al.*, 2016).

The genome of strain KCOM 3500 was composed of one contig, 2,778,422 bp in length. The G + C content of the genome was 38.1% (Table 1). A total of 2,507 protein-coding sequences, 6 rRNAs, and 44 tRNAs were annotated (Table 1). To confirm the classification of strain KCOM 3500 at the species level, homologous analysis of the genome sequence was performed using the programs of average nucleotide identity (ANI) (OrthoANI, Lee *et al.*, 2016) and genome-to-genome distance calculation (Meier-Kolthoff *et al.*, 2013). The OrthoANI and GGD values between the genome sequence of strain KCOM 3500 and that of *T. denticola* ATCC 35405<sup>T</sup> were 97.26% (Fig. 1B) and 74.3% (71.3–77.1%), respectively. However, the OrthoANI and GGD values between strain KCOM 3500 and each type strain of the other two *Treponema* sp. closest to *T. denticola* were below 95% and 70%, respectively. These result show that strain KCOM 3500 belonged to *T. denticola*.

The genome sequence of strain KCOM 3500 contained virulence-related genes: a dentilisin complex serine proteinase (subunit PrtP [OE909\_RS09950], subunit PrcA [OE909\_RS09955], subunit PrcB [OE909\_RS09960]), four hemolysin family proteins (OE909\_RS07900, OE909\_RS00115, OE909\_RS00120, OE909\_RS03035), twenty two chemotaxis proteins (CheA [OE909\_RS06755], CheX [OE909\_RS06745], CheW

[OE909\_RS06750, OE909\_RS06285], OE909\_RS07260, OE909\_RS07710, OE909\_RS08655, OE909\_RS08865, OE909\_RS09550, OE909\_RS10435, OE909\_RS11165, OE909\_RS11765, OE909\_RS12215, OE909\_RS12280, OE909\_RS00205, OE909\_RS00240, OE909\_RS00245, OE909\_RS00635, OE909\_RS01595, OE909\_RS01990, OE909\_RS02195, OE909\_RS03280), tetracycline resistance ribosomal protection protein Tet(32) (OE909\_RS01370). It also contained a type II and III secretion system protein (OE909\_RS04395), forty toxin-antitoxin system proteins (OE909\_RS06925, OE909\_RS06930, OE909\_RS07290, OE909\_RS07295, OE909\_RS08330, OE909\_RS09410, OE909\_RS10270, OE909\_RS10320, OE909\_RS10325, OE909\_RS11025, OE909\_RS11030, OE909\_RS11105, OE909\_RS11175, OE909\_RS11180, OE909\_RS11260, OE909\_RS11355, OE909\_RS11360, OE909\_RS12345, OE909\_RS12520, OE909\_RS12530, OE909\_RS00025, OE909\_RS00030, OE909\_RS00790, OE909\_RS00795, OE909\_RS00895, OE909\_RS00900, OE909\_RS01925, OE909\_RS03330, OE909\_RS03335, OE909\_RS03360, OE909\_RS03910, OE909\_RS04650, OE909\_RS04735, OE909\_RS04740, OE909\_RS04745, OE909\_RS04750, OE909\_RS05325, OE909\_RS05970, OE909\_RS06030, OE909\_RS06035), two paired two-component systems (OE909\_RS10350/OE909\_RS10355, OE909\_RS12115/OE909\_RS12120), and an unmatched sensor histidine kinase (OE909\_RS02780), five unmatched regulatory proteins (OE909\_RS04540, OE909\_RS11960, OE909\_RS12040, OE909\_RS01735, OE909\_RS04005), and phage-related proteins: phage protease (OE909\_RS01745), phage virion morphogenesis protein (OE909\_RS01765), phage tail sheath protein (OE909\_RS01765), phage tail tube protein (OE909\_RS01780), phage tail tape measure protein (OE909\_RS01790), and phage GP 46 family protein (OE909\_RS01810). The results of genes analysis in whole genome sequence of strain KCOM 3500 could offer some clues in understanding the mechanism of the pathogenicity of *T. denticola* in periodontal diseases.

#### Nucleotide sequence accession number

This whole genome sequence of strain KCOM 3500 was deposited in GenBank under the accession number CP109619.

## 적 요

*Treponema denticola*는 절대 혐기성, 그람 음성, 아포를 생성하지 않고 및 비운동성 나선모양의 세균이다. 이 세균 종은 치주염의 주요한 원인균 중이며 알츠하이머 질환과 심혈관계 질환과 같은 전신질환과도 연관이 있다. *Treponema denticola* KCOM 3500 (= ChDC P019-Td1) 균주는 사람 만성치주염 병소 부위의 치은연하 치면세균막에서 분리되었다. KCOM 3500 균주 전장 유전체는 2,778,422 bp로 구성되어 있고, 2,507개의 단백질 코딩 시퀀스, 6개 rRNAs 및 44개 tRNAs를 함유하고 있었다. 이 균주의 G+C 함량은 38.1%였다. 전장 유전체에는 강력한 단백분해효소인 dentilisin 복합 세린 단백분해효소를 함유하고 있었다.

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## Conflict of Interest

The authors have no conflict of interest to report.

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