



Title	Phylogenetic relationships between <i>Treponema putidum</i> and <i>Treponema denticola</i> isolates
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Citation	The 2012 PER/IADR Congress, Helsinki, Finland, 12-15 September 2012.
Issued Date	2012
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PHYLOGENETIC RELATIONSHIPS BETWEEN *TREPONEMA PUTIDUM* AND *TREPONEMA DENTICOLA* ISOLATES

Treponema putidum is a recently identified species of oral spirochete bacteria, first isolated from human periodontitis lesions and acute necrotizing ulcerative gingivitis sites in patients from China. It is an obligately anaerobic; motile; spiral-shaped; proteolytic and asaccharolytic spirochete. Its 16S rRNA gene sequence is closely-related to that of *Treponema denticola*, placing it within oral treponeme phylogroup two.

OBJECTIVES: To use a multi-gene sequencing approach to characterize genetic diversity within *T. putidum* strains, to establish their phylogenetic relationships with other species of human and non-human treponemes.

METHODS: Sets of primers based on *T. denticola* gene sequences, were screened for their ability to PCR amplify gene fragments from five *T. putidum* strains (OMZ 758 (type), 844, 846, 847, 848) as well as several dog and human *T. denticola* isolates (generously supplied by Dr. Chris Wyss). Individual and concatenated gene sequences were analyzed using various computational evolutionary biology approaches, including Bayesian inference and Maximum Likelihood (ML) analysis, to establish intra- and inter-species phylogenetic relationships.

RESULTS: The sequences of the 16S rRNA, *pyrH* and *recA* genes were successfully determined for all strains. The *T. putidum* isolates shared a common ancestor distinct from that of *T. denticola*, *Treponema pallidum* and *Treponema phagedenis*. The dog and human *T. denticola* isolates formed distinct clusters, although some strains had anomalous phylogenetic patterns e.g. *T. denticola* ATCC 700768 (OMZ830, PROS strain). Results from preliminary attempts to use *pyrH* primers to analyze oral treponeme diversity in clinical periodontitis samples are discussed.

CONCLUSIONS: The sequence of the highly-conserved *pyrH* gene is a robust and highly-discriminatory genetic marker for the differentiation and taxonomic characterization of oral treponeme isolates.