


# Draft Genome Sequences of Three Clinical Isolates of *Tannerella forsythia* Isolated from Subgingival Plaque from Periodontitis Patients in the United States

Graham P. Stafford,<sup>a</sup> Roy R. Chaudhuri,<sup>b</sup> Violet Haraszthy,<sup>c</sup> Valentin Friedrich,<sup>d</sup> Christina Schäffer,<sup>d</sup> Angela Ruscitto,<sup>c</sup> Kiyonobu Honma,<sup>c</sup>  Ashu Sharma<sup>c</sup>

Integrated Biosciences Group, School of Clinical Dentistry, University of Sheffield, Sheffield, United Kingdom<sup>a</sup>; Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield, United Kingdom<sup>b</sup>; Department of Oral Biology, University at Buffalo, SUNY, Buffalo, New York, USA<sup>c</sup>; Department of NanoBiotechnology, NanoGlycobiology Unit, Universität für Bodenkultur Wien, Muthgasse, Vienna, Austria<sup>d</sup>

**We report the genome sequences of three clinical isolates of *Tannerella forsythia* from the subgingival plaque of periodontitis patients attending clinics at the School of Dental Medicine, University at Buffalo. The availability of these genome sequences will aid the understanding of the pathogenesis of periodontitis.**

Received 21 September 2016 Accepted 7 October 2016 Published 1 December 2016

**Citation** Stafford GP, Chaudhuri RR, Haraszthy V, Friedrich V, Schäffer C, Ruscitto A, Honma K, Sharma A. 2016. Draft genome sequences of three clinical isolates of *Tannerella forsythia* isolated from subgingival plaque from periodontitis patients in the United States. *Genome Announc* 4(6):e01286-16. doi:10.1128/genomeA.01286-16.

**Copyright** © 2016 Stafford et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Ashu Sharma, sharmaa@buffalo.edu.

Periodontitis is a common condition in humans, estimated to affect approximately 20% of those 35 to 44 years old globally (1) and 47% of the U.S. population (2). Periodontitis describes a group of conditions characterized by chronic inflammation of the gingiva, recession of gums, degradation of the supporting structures of the tooth, including the periodontal ligament and alveolar bone, and formation of periodontal pockets that act as a niche for colonizing bacteria (3). It is increasingly linked with systemic sequelae such as rheumatoid arthritis (4, 5), diabetes (6), and cardiovascular disease (7) and represents a major global health burden.

Unlike other conditions, periodontitis is considered a polymicrobial disease with the presence of certain organisms representative of a generalized dysbiosis contributing to the observed symptoms (8). This smaller group of periodontal pathogens is often termed the “red complex” after the pioneering work of Socransky and colleagues (9). Within this red complex of bacteria, *Tannerella forsythia* has long been an understudied organism, mainly due to difficulties in cultivation and isolation owing to fastidious growth requirements, such as requirement for *N*-acetylmuramic acid (MurNAc) (10).

We report here the draft genome sequences of three novel *T. forsythia* strains, isolated from the subgingival plaque of patients attending the clinic at the School of Dental Medicine, University at Buffalo, in 2013. Briefly, plaque samples from periodontal pockets >5 mm were serially diluted and plated on tryptic soy

blood agar plus MurNAc plates (11) and incubated anaerobically (37°C), before sialidase-positive colonies were picked and grown in broth cultures and gDNA was isolated. Colonies with a 16S rDNA sequence identifying them as strains of *T. forsythia* via comparison to ATCC 43037 were subsequently passaged and maintained on agar. Three strains were isolated and named UB4, UB20, and UB22.

DNA was extracted using Promega Wizard kits before sequencing on the Illumina platform using 2 × 300-bp paired-end sequencing either commercially at MrDNA, Shallowater, TX, USA (UB22), or via MicrobesNG, United Kingdom (UB4 and UB20). Contigs were assembled and scaffolded using SPAdes version 3.8.0 with the options “-careful, -cov\_cutoff 10,” followed by automated annotation using Prokka (12). The scaffolds were re-ordered and reoriented based on NUCmer (13) comparisons with the published *T. forsythia* 92A2 genome sequence (CP003191), and the annotations were manually inspected and refined using ACT (14).

General properties of the genomes are summarized in Table 1. The genomes are generally well conserved with >90% similarity for most genes and very similar GC contents. Recent work has revealed several virulence determinants in this organism (8, 15, 16). First, a TLR-2 activating surface protein called BspA present in all three strains is well conserved in the C-terminal region. Furthermore, the sialic acid utilization *nan* gene cluster can be

**TABLE 1** Metadata for *T. forsythia* strains isolated from subgingival plaque from patients in Buffalo, NY, USA

Strain name	Assembly accession no.	Run accession no.	Source tissue	Total assembly size (bp)	<i>N</i> <sub>50</sub>	G+C content (%)	No. of scaffolds
UB4	<a href="https://ncbi.nlm.nih.gov/assembly/FMMN01000000/">FMMN01000000</a>	ERR1633772	Subgingival plaque	3,232,117	136,594	47.20	71
UB20	<a href="https://ncbi.nlm.nih.gov/assembly/FMMM01000000/">FMMM01000000</a>	ERR1633773	Subgingival plaque	3,252,817	140,079	47.05	92
UB22	<a href="https://ncbi.nlm.nih.gov/assembly/FMML01000000/">FMML01000000</a>	ERR1633774	Subgingival plaque	3,272,088	93,893	47.07	97

found in all strains (17), as well as genes encoding the S-layer (*tfsAB*) and a novel MurNAc uptake system (18).

The availability of these draft genome sequences will improve understanding of *T. forsythia* and aid in forging new treatments for globally important periodontitis disease.

**Accession number(s).** The accession numbers for the draft sequences of the three *T. forsythia* strains and scaffold sequences deposited in the European Nucleotide Archive (ENA) and NCBI databases are listed in Table 1.

## ACKNOWLEDGMENTS

Genome sequencing and bioinformatics were performed by MicrobesNG (<http://microbesng.uk>), which is supported by the BBSRC (grant number BB/L024209/1) and USPH grant DE14749 to A.S. V.F. was funded by Austrian Science Fund FWF project W-1224 (PhD Programme Biomolecular Technology of Proteins).

## FUNDING INFORMATION

This work, including the efforts of Roy R. Chaudhuri, was funded by the Biotechnology and Biological Sciences Research Council (BBSRC) (BB/L024209/1). This work, including the efforts of Ashu Sharma, was funded by HHS | U.S. Public Health Service (USPHS) (DE14749).

## REFERENCES

1. WHO. 2012. Fact sheet N318: oral health. WHO, Geneva, Switzerland.
2. Eke PI, Dye BA, Wei L, Thornton-Evans GO, Genco RJ, CDC Periodontal Disease Surveillance Workgroup. 2012. Prevalence of periodontitis in adults in the United States: 2009 and 2010. *J Dent Res* 91:914–920. <http://dx.doi.org/10.1177/0022034512457373>.
3. Hajishengallis G, Darveau RP, Curtis MA. 2012. The keystone-pathogen hypothesis. *Nat Rev Microbiol* 10:717–725. <http://dx.doi.org/10.1038/nrmicro2873>.
4. Kaur S, White S, Bartold PM. 2013. Periodontal disease and rheumatoid arthritis: a systematic review. *J Dent Res* 92:399–408. <http://dx.doi.org/10.1177/0022034513483142>.
5. Moen K, Brun JG, Valen M, Skartveit L, Eribe EK, Olsen I, Jonsson R. 2006. Synovial inflammation in active rheumatoid arthritis and psoriatic arthritis facilitates trapping of a variety of oral bacterial DNAs. *Clin Exp Rheumatol* 24:656–663.
6. Borgnakke WS, Ylöstalo PV, Taylor GW, Genco RJ. 2013. Effect of periodontal disease on diabetes: systematic review of epidemiologic observational evidence. *J Clin Periodontol* 40(suppl 14):S135–S152. <http://dx.doi.org/10.1111/jcpe.12080>.
7. Schaefer AS, Richter GM, Groessner-Schreiber B, Noack B, Nothnagel M, El Mokhtari N-E, Loos BG, Jepsen S, Schreiber S. 2009. Identification of a shared genetic susceptibility locus for coronary heart disease and periodontitis. *PLoS Genet* 5:e1000378. <http://dx.doi.org/10.1371/journal.pgen.1000378>.
8. Douglas CW, Naylor K, Phansopa C, Frey AM, Farmilo T, Stafford GP. 2014. Physiological adaptations of key oral bacteria. *Adv Microb Physiol* 65:257–335. <http://dx.doi.org/10.1016/bs.ampbs.2014.08.005>.
9. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL. 1998. Microbial complexes in subgingival plaque. *J Clin Periodontol* 25: 134–144. <http://dx.doi.org/10.1111/j.1600-051X.1998.tb02419.x>.
10. Wyss C. 1989. Dependence of proliferation of *Bacteroides forsythus* on exogenous *N*-acetylmuramic acid. *Infect Immun* 57:1757–1759.
11. Roy S, Honma K, Douglas CW, Sharma A, Stafford GP, Douglas CWI, Sharma A, Stafford GP. 2011. Role of sialidase in glycoprotein utilisation by *Tannerella forsythia*. *Microbiology* 157:3195–3202. <http://dx.doi.org/10.1099/mic.0.052498-0>.
12. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30:2068–2069. <http://dx.doi.org/10.1093/bioinformatics/btu153>.
13. Kurtz S, Phillippy A, Delcher AL, Smoot M, Shumway M, Antonescu C, Salzberg SL. 2004. Versatile and open software for comparing large genomes. *Genome Biol* 5:R12. <http://dx.doi.org/10.1186/gb-2004-5-2-r12>.
14. Carver TJ, Rutherford KM, Berriman M, Rajandream MA, Barrell BG, Parkhill J. 2005. ACT: the Artemis comparison tool. *Bioinformatics* 21: 3422–3423. <http://dx.doi.org/10.1093/bioinformatics/bti553>.
15. Sharma A. 2010. Virulence mechanisms of *Tannerella forsythia*. *Periodontol* 2000 54:106–116. <http://dx.doi.org/10.1111/j.1600-0757.2009.00332.x>.
16. Posch G, Sekot G, Friedrich V, Megson ZA, Koerdt A, Messner P, Schäffer C. 2012. Glycobiology aspects of the periodontal pathogen *Tannerella forsythia*. *Biomolecules* 2:467–482. <http://dx.doi.org/10.3390/biom2040467>.
17. Stafford G, Roy S, Honma K, Sharma A. 2011. Sialic acid, periodontal pathogens and *Tannerella forsythia*: stick around and enjoy of the feast! *Mol Oral Microbiol* 26:11–22. <http://dx.doi.org/10.1111/j.2041-1014.2011.00630.x>.
18. Ruscitto A, Hottmann I, Stafford GP, Schäffer C, Mayer C, Sharma A. 2016. Identification of a novel *N*-acetylmuramic acid (MurNAc) transporter in *Tannerella forsythia*. *J Bacteriol* 198:3119–3125. <http://dx.doi.org/10.1128/JB.00473-16>.