Treponema ruminis sp. nov., a spirochaete isolated from the bovine rumen.

Running title: A novel treponeme isolated from the bovine rumen.

Kerry Newbrook[1#], Gareth J. Staton[1], Simon R. Clegg[1], Richard J. Birtles[2], Stuart D. Carter[1], Nicholas J. Evans[1]

[1]Department of Infection Biology, Institute of Infection and Global Health, University of Liverpool, Liverpool Science Park Innovation Centre 2, 146 Brownlow Hill, Liverpool, United Kingdom, L3 5RF.

[2]School of Environment and Life Sciences, University of Salford, Peel Building, Salford, Greater Manchester, United Kingdom, M5 4WT.

[#]Corresponding author: Kerry Newbrook, Department of Infection Biology, Institute of Infection and Global Health, University of Liverpool, Liverpool Science Park Innovation Centre 2, 146 Brownlow Hill, Liverpool, United Kingdom, L3 5RF, kerrynewbrook@btinternet.com Tel: +44 151 794 4755.

Keywords: *Treponema*, Spirochaete, Bovine, Rumen.

Subject category: "NEW TAXA: Other bacteria".

Word count: 1,665.

Genbank accession numbers: The GenBank accession number for the 16S rRNA gene sequence of *Treponema ruminis* is GU566698. The GenBank accession number for the recombinase A (*recA*) gene sequence of *Treponema ruminis* is KX261205.

Abbreviations. GI, gastrointestinal; *recA*, recombinase A; RS, rabbit serum.

Abstract

A novel bacterium, Ru1^T, was encountered during a survey of spirochaetes living in the gastrointestinal (GI) tract of ruminants. Comparative analysis of 16S rRNA gene sequence data indicated that Ru1^T clustered within the *Treponema* genus but shared at most 86.1% sequence similarity with other recognised *Treponema* species. Further phylogenetic analysis based on partial recombinase A (*recA*) gene sequence comparisons, together with phenotypic characterisation, also demonstrated the divergence of Ru1^T from other recognised *Treponema* species. Microscopically, Ru1^T appeared as a very small, highly motile, helical spirochaete with four periplasmic flagella. It exhibited C8 esterase lipase, leucine arylamidase, β-galactosidase and β-glucosidase activity. A distinctive, serum-independent growth pattern was also observed, characterised by colonies with an absence of the local haemolysis that is typical of many pathogenic treponemes. On the basis of these data, Ru1^T is considered to represent a new *Treponema* species for which the name *Treponema ruminis* sp. nov. is proposed. The type strain of *Treponema ruminis* is Ru1^T (=DSM 103462^T=NCTC 13847^T).

Comment [CS1]: ??? isolated?

Main Text

Treponema species are fastidious, highly motile, helical, anaerobic microorganisms of the spirochaete phylum which have been identified within the gastrointestinal (GI) tract, oral cavity and genital areas of animals, humans and insects (Smirbert, 1984). Some treponemes are associated with infectious diseases including digital dermatitis, human periodontal disease, pinta, yaws and the venereal infection, syphilis (Choi *et al.*, 1997; Dewhirst *et al.*, 2000; Engelkens *et al.*, 1991; Mitjà *et al.*, 2013; Radolf *et al.*, 2006) but others are commensal symbionts living in the GI tract of animals and insects.

Due to their fastidious nature, only a handful of GI treponemes have been characterised. *Treponema succinifaciens*, *Treponema porcinum* and *Treponema berlinense* were isolated from porcine GI contents (Cwyk & Canale-Parola, 1979; Nordhoff *et al.*, 2005). Similarly, *Treponema isoptericolens*, *Treponema azotonutricium* and *Treponema primitia* have been isolated from the digestive tract of termites (Dröge *et al.*, 2008; Graber *et al.*, 2004). Metagenomic studies have identified a diverse variety of spirochaetes within the bovine rumen (Edwards *et al.*, 2004; Paster & Canale-Parola, 1982; Tajima *et al.*, 1999; Zinicola *et al.*, 2015) and although several have been successfully isolated (Evans *et* al., 2011; Ziolecki, 1979; Ziolecki & Wojciechowicz, 1980), only two have been formally proposed as novel treponeme taxa, namely *Treponema bryantii* and *Treponema saccharophilum* (Paster & Canale-Parola, 1985; Stanton & Canale-Parola, 1980).

A recent study aimed to isolate and characterise spirochaetes from the GI tract of Holstein-Friesian cattle in the United Kingdom (UK) for comparison with bovine digital dermatitis treponemes (Evans *et al.*, 2011). Seven 16S rRNA gene sequence variants were obtained that were found to cluster into four novel phylotypes within the *Treponema* genus. Each phylotype shared less than 97% 16S rRNA gene sequence identity to extant *Treponema* species, suggesting these isolates represent novel taxa. In the present study we further characterised one of these phylotypes, represented by strain Ru1^{T}, and on the basis of this characterisation propose it as a novel *Treponema* species.

Strain Ru1^{T} was isolated at slaughter from the rumen contents of a Holstein-Friesian bull from a UK dairy farm, as previously described (Evans *et al.*, 2011). The isolate was initially grown anaerobically (85% nitrogen, 10% hydrogen, 5% carbon dioxide) at 36°C in oral treponeme enrichment broth (Anaerobe Systems, Morgan Hill, USA) supplemented with 10% (v/v) rabbit serum (RS; GE Healthcare Life Sciences, Buckinghamshire, UK). However, after isolation the spirochaete did not require RS for further growth and thereafter was successfully and routinely subcultured daily in the absence of serum. Under phase contrast microscopy, the cells exhibited morphological characteristics which are typical of many treponemes in culture including rotational and translational movement, jerky flexing and high motility. The isolate was successfully stored at -80°C in growth medium containing 10% (v/v) glycerol. Cells were additionally subcultured onto unsupplemented fastidious

anaerobe agar (LabM, Bury, UK) plates. Following anaerobic incubation for 10 days, colonies appeared which were circular, translucent, convex and pinprick-sized with a diameter of between 0.2-0.5mm. The colonies exhibited no metallic sheen or local haemolysis and were morphologically very different to those reported for several other taxonomically appraised GI treponemes, which are typically much larger in size. For example, *T. succinifaciens* ATCC 33096^T and *T. saccharophilum* ATCC 43261^T form spherical, opaque colonies with a diameter of 4-8mm and 3-4mm respectively, whereas colonies of *T. porcinum* ATCC BAA-908^T and *T. berlinense* ATCC BAA-909^T exhibit a 1-2mm diameter and are irregular and greyish in colouration (Cwyk & Canale-Parola, 1979; Nordhoff *et al.*, 2005; Paster & Canale-Parola, 1985).

Genomic DNA was extracted from the cultured isolate for subsequent PCR amplification and sequencing of the 16S rRNA gene product, as previously described (Evans *et al.*, 2008; Evans *et al*., 2011) and the near-complete 16S rRNA gene sequence (1309bp) of Ru1^T was aligned with the 16S rRNA gene sequences of extant *Treponema* species in the Bioedit Sequence Alignment Editor using CLUSTAL W (Hall, 2013; Thompson *et al.*, 1994). Ru1^T shared highest sequence similarity (86.1%) with *T. porcinum* ATCC BAA-908^T, a spirochaete isolated from porcine GI tract contents (Nordhoff *et al*., 2005). This level of sequence similarity is well below the proposed threshold for species delineation (Stackebrandt & Goebel, 1994). Phylogeny was inferred from this alignment, using ModelTest software in the TOPALi v2 program to predict the best-fit evolutionary model (Milne *et al.*, 2009). The Tamura-Nei model was subsequently used to produce a bootstrapped maximum-likelihood tree based upon 10,000 reiterations, as implemented in MEGA 6.0 (Tamura *et al.*, 2013; Tamura & Nei, 1993). Phylogenetic reconstruction revealed that Ru1^T formed a distinct phylotype within a wider, deep-branched region of porcine and bovine GI tract treponemes (Figure 1). Ru1^T clustered specifically with *T. succinifaciens* ATCC 33096^T and *T. saccharophilum* ATCC 43261^T (sharing 85.8% and 84.1% 16S rRNA gene sequence identity respectively) and then with *T. porcinum* ATCC BAA-908^T. Whilst clustering with these GI tract treponemes, Ru1^T had diverged markedly from them such that they were separated by phylogenetic distances akin to those observed among extant *Treponema* species.

A novel degenerate PCR assay was developed and optimised for amplification of the recombinase A (*recA*) gene from Ru1^T, with this gene having recently been used in the phylogenetic typing of treponeme isolates (Clegg *et al.,* 2016). The PCR assay incorporated the primer pair *recA* forward (5'-GCAACYTTGTTCTTTACR-3') and *recA* reverse (5'-GAAATGTACGGTCCYGAA-3'), designed following the alignment of *recA* gene sequences from relevant *Treponema* of the bovine and porcine GI tract phylogenetic cluster (Evans *et al.,* 2011) using CLUSTAL W within the Bioedit Sequence Alignment Editor. Genomic treponemal DNA (1µl) was incorporated into a 25µl PCR master mix containing 10µM of each degenerate primer, 20mM dNTP mix (5mM each of dATP, dCTP, dGTP, dTTP; Thermo Scientific™, Hemel Hempstead, UK) and *Taq* DNA polymerase according to the manufacturer's instructions (Qiagen, Manchester, UK). Mixes were subjected to a thermal cycle of 95°C for 5 minutes; 40 cycles of 94°C for 1 minute, 49.1°C for 3 minutes, 72°C for 3 minutes; 72°C for 7 minutes. The presence of amplification products was verified by agarose gel electrophoresis, and, when present, these were purified and both strands were sequenced commercially (Source BioScience, Nottingham, UK). A partial (479bp) *recA* alignment of Ru1^T and *Treponema* species was generated in the Bioedit Sequence Alignment Editor using CLUSTAL W. Treponemal *recA* gene sequences differed from one another markedly. The mean *recA* gene sequence similarity between extant *Treponema* species was 64.3% (range 56.3% to 93.8%) whilst the mean intra-species *recA* gene sequence similarities for two recognised *Treponema* spp. (with *recA* data available for a range of isolates) were calculated as 99.1% and 97.4% (for *Treponema medium* and *Treponema pedis* respectively) (Clegg *et al.,* 2016). The *recA* gene sequence of Ru1^T was most similar to that of *T. succinifaciens* ATCC 33096^T (76.8%). It also shared 75.3% *recA* gene sequence similarity with *T. brennaborense* CIP 105900^T (Schrank *et al.,* 1999) and *Treponema socranskii* subsp. *paredis* ATCC 35535^T (Smibert *et al.,* 1984). A phylogeny was derived from the *recA* alignment using the Tamura-Nei model based upon 10,000 reiterations, as implemented in MEGA 6.0 (Tamura & Nei, 1993). In this phylogeny (Figure 2), Ru1^T was again a distinct phylotype among bovine and porcine treponeme isolates.

The API® ZYM system (bioMérieux, Lyon, France) was used to generate an enzyme activity profile for Ru1^T, as reported previously (Evans *et al.*, 2011). Positive enzyme activity was detected for C8 esterase lipase, leucine arylamidase, β-galactosidase and β-glucosidase. However, no enzyme activity was detected for alkaline phosphatase, C4 esterase, C14 lipase, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, acid phosphatase, naphtholphohydrolase, α-galactosidase, β-glucuronidase, α-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Among *Treponema* species, Ru1^T was found to have a unique API® ZYM profile (Table 1).

The morphology of Ru1^T was determined directly from liquid culture by transmission electron microscopy, as reported previously (Demirkan *et al.*, 2006; Evans *et al.*, 2011). Whilst sharing the common morphological characteristics of other *Treponema* species, Ru1^T could be distinguished on the basis of being approximately 5-9µm in length, 0.4-0.5µm in width and having between 3-5 regular coils. Each cell possessed 4 periplasmic flagella.

Based upon the comparative data presented in this study, strain Ru1^T is considered to represent a novel species within the *Treponema* genus, for which the name *Treponema ruminis* sp. nov. is proposed.

Description of *Treponema ruminis* sp. nov. *Treponema ruminis* (ru'mi.nis. L. neut. gen. n. *ruminis*, of the rumen). Anaerobic, gram-negative, helically coiled, motile and very small-sized treponemes. Cells are approximately 5-9µm long and 0.40-0.50µm wide, have between 3-5 even windings and each have 4 periplasmic flagella. Cells typically reach optimal growth following anaerobic incubation at 36°C for 1 day within oral treponeme enrichment broth without serum supplementation. Cells exhibit translational movement, rotation and jerky flexing in culture and typically sediment towards the bottom of the tube. Circular, translucent, convex, pinprick-sized colonies of 0.20-0.50mm diameter are observed after 10 days when streaked onto unsupplemented fastidious anaerobe agar plates. Colonies do not have a metallic sheen or exhibit local hemolysis. Cells can be stored at -80°C

in growth medium containing 10%(v/v) glycerol. The API® ZYM system identified enzyme activity for C8 esterase lipase, leucine arylamidase, β-galactosidase and β-glucosidase, while detecting no activity for alkaline phosphatase, C4 esterase, C14 lipase, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, acid phosphatase, naphtholphohydrolase, α-galactosidase, β-glucuronidase, α-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase.

The type strain, Ru1^{T} (=DSM 103462^{T}=NCTC 13847^{T}), was isolated from the rumen contents of a Holstein-Friesian bull from a Cheshire farm in the UK.

Acknowledgements

This work was funded by a BBSRC DTP studentship, awarded to Kerry Newbrook and supervisors Nicholas J. Evans and Stuart D. Carter, and a BBSRC New Investigator Award (BB/K009443/1) awarded to Nicholas J. Evans.

We would like to acknowledge Professor Aharon Oren for his kind help in identifying the correct nomenclature.

Furthermore, we would like to acknowledge the Leibniz-Institut Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ; Braunschweig, Germany) and the National Collection of Type Cultures (NCTC; South Mimms, UK) for their help with strain deposition.

References

Choi, B.K., Nattermann, H., Grund, S., Haider, W. & Göbel, U.B. (1997). Spirochetes from digital dermatitis lesions in cattle are closely related to treponemes associated with human periodontitis. *Int J Syst Bacteriol* **47(1)**, 175-181.

Clegg, S.R., Carter, S.D., Birtles, R.J., Brown, J.M., Hart, C.A. & Evans, N.J. (2016). Multilocus sequence typing of pathogenic treponemes isolated from cloven-hoofed animals and comparison to treponemes isolated from humans. *Appl Environ Microbiol* **82(15)**, 4523-4536.

Cwyk, W.M. & Canale-Parola, E. (1979). *Treponema succinifaciens* sp. nov., an anaerobic spirochete from the swine intestine. *Arch Microbiol* **122(3)**, 231-239.

Demirkan, I., Williams, H.F., Dhawi, A., Carter, S.D., Winstanley, C., Bruce, K.D. & Hart, C.A. (2006). Characterization of a spirochaete isolated from a case of bovine digital dermatitis. *J Appl Microbiol* **101(4)**, 948-955.

Dewhirst, F.E., Tamer, M.A., Ericson, R.E., Lau, C.N., Levanos, V.A., Boches, S.K., Galvin, J.L. & Paster, B.J. (2000). The diversity of periodontal spirochetes by 16S rRNA analysis. *Oral Microbiol Immunol* **15(3)**, 196-202.

Dröge, S., Rachel, R., Radek, R. & König, H. (2008). *Treponema isoptericolens* sp. nov., a novel spirochaete from the hindgut of the termite *Incisitermes tabogae. Int J Syst Evol Microbiol* **58(5)**, 1079-1083.

Edwards, J.E., McEwan, N.R., Travis, A.J. & Wallace, R.J. (2004). 16S rDNA library-based analysis of ruminal bacterial diversity. *Antonie van Leeuwenhoek* **86(3)**, 263-281.

Engelkens, H.J., Niemel, P.L., van der Sluis, J.J., Megheus, A. & Stolz, E. (1991). Endemic treponematoses. Part II. Pinta and endemic syphilis. *Int J Dermatol* **30(4)**, 231-238.

Evans, N.J., Brown, J.M., Demirkan, I., Murray, R.D., Vink, W.D., Blowey, R.W., Hart, C.A. & Carter, S.D. (2008). Three unique groups of spirochetes isolated from digital dermatitis lesions in UK cattle. *Vet Microbiol* **130(1-2)**, 141-150.

Evans, N.J., Brown, J.M., Demirkan, I., Murray, R.D., Birtles, R.J., Hart, C.A. & Carter, S.D. (2009). *Treponema pedis* sp. nov., a spirochaete isolated from bovine digital dermatitis lesions. *Int J Syst Evol Microbiol* **59(Pt5)**, 987-991.

Evans, N.J., Brown, J.M., Murray, R.D., Getty, B., Birtles, R.J., Hart, C.A. & Carter, S.D. (2011). Characterization of novel bovine gastrointestinal tract *Treponema* isolates and comparison with bovine digital dermatitis treponemes. *Appl Environ Microbiol* **77(1)**, 138-147.

Graber, J.R., Leadbetter, J.R. & Breznak, J.A. (2004). Description of *Treponema azotonutricium* sp. nov. and *Treponema primitia* sp. nov., the first spirochetes isolated from termite guts. *Appl Environ Microbiol* **70(3)**, 1315-1320.

Hall, T. (2013). BioEdit: Biological sequence alignment editor for Win95/98/NT/2K/XP/7. Carlsbad, CA: Ibis Biosciences. Available from http://www.mbio.ncsu.edu/bioedit/bioedit.html (Accessed 08 January 2016).

Milne, I., Lindner, D., Bayer, M., Husmeier, D., McGuire, G., Marshall, D.F. & Wright, F. (2009). TOPALi v2: a rich graphical interface for evolutionary analyses of multiple alignments on HPC clusters and multi-core desktops. *Bioinformatics* **25(1)**, 126-127.

Mitjà, O., Asiedu, K. & Mabey, D. (2013). Yaws. *Lancet* **381**, 763-773.

Nordhoff, M., Taras, D., Macha, M., Tedin, K., Busse, H.J. & Wieler, L.H. (2005). *Treponema berlinense* sp. nov. and *Treponema porcinum* sp. nov., novel spirochaetes isolated from porcine faeces. *Int J Syst Evol Microbiol* **55(4)**, 1675-1680.

Paster, B.J. & Canale-Parola, E. (1982). Physiological diversity of rumen spirochetes. *Appl Environ Microbiol* **43(3)**, 686-693.

Paster, B.J. & Canale-Parola, E. (1985). *Treponema saccharophilum* sp. nov., a large pectinolytic spirochete from the bovine rumen. *Appl Environ Microbiol* **50(2)**, 212-219.

Radolf, J.D., Hazlett, K.R.O. & Lukehart, S.A. (2006). Pathogenesis of syphilis. In *Pathogenic Treponema: Molecular and Cellular Biology*, pp. 197-236. Edited by J.D. Radolf & S.A. Lukehart. Norfolk: Caister Academic Press.

Schrank, K., Choi, B.K., Grund, S., Moter, A., Heuner, K., Nattermann, H. & Göbel, U.B. (1999). *Treponema brennaborense* sp. nov., a novel spirochaete isolated from a dairy cow suffering from digital dermatitis. *Int J Syst Bacteriol* **49(Pt1)**, 43-50.

Smirbert, R.M. (1984). Genus III *Treponema.* In *Bergey's Manual of Systematic Bacteriology*, pp. 49-57. Volume 1. Edited by N.R. Krieg & J.G. Holt. Baltimore: Williams and Wilkins.

Smirbert, R.M., Johnson, J.L., Ranney, R.R. (1984). *Treponema socranskii* sp. nov., *Treponema socranskii* subsp. *socranskii* subsp. nov., *Treponema socranskii* subsp. *buccale* subsp. nov., and *Treponema socranskii* subsp. *paredis* subsp. nov. Isolated from the human periodontia. *Int J Syst Bacteriol* **34(4)**, 457-462.

Stackebrandt, E. & Goebel, B.M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44(4)**, 846-849.

Stanton, T.B. & Canale-Parola, E. (1980). *Treponema bryantii* sp. nov., a rumen spirochete that interacts with cellulolytic bacteria. *Arch Microbiol* **127(2)**, 145-156.

Tajima, K., Aminov, R.I., Nagamine, T., Ogata, K., Nakamura, M., Matsui, H. & Benno, Y. (1999). Rumen bacterial diversity as determined by sequence analysis of 16S rDNA libraries. *FEMS Microbiol Ecol* **29(2)**, 159-169.

Tamura, K. & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol Biol Evol* **10(3)**, 512-526.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Mol Biol Evol* **30(12)**, 2725-2729.

Thompson, J.D., Higgins, D.G. & Gibson, T.J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22(22)**, 4673-4680.

Wyss, C., Choi, B.K., Schüpbach, P., Guggenheim, B. & Göbel, U.B. (1996). *Treponema maltophilum* sp. nov., a small oral spirochete isolated from human periodontal lesions. *Int J Syst Bacteriol* **46(3)**, 745-752.

Wyss, C., Choi, B.K., Schüpbach, P., Guggenheim, B. & Göbel, U.B. (1997). *Treponema amylovorum* sp. nov., a saccharolytic spirochete of medium size isolated from an advanced human periodontal lesion. *Int J Syst Bacteriol* **47(3)**, 842-845.

Wyss, C., Choi, B.K., Schüpbach, P., Moter, A., Guggenheim, B. & Göbel, U.B. (1999). *Treponema lecithinolyticum* sp. nov., a small saccharolytic spirochaete with phospholipase A and C activities associated with periodontal diseases. *Int J Syst Bacteriol* **49(Pt4)**, 1329-1339.

Wyss, C., Dewhirst, F.E., Gmür, R., Thurnheer, T., Xue, Y., Schüpbach, P., Guggenheim, B. & Paster, B.J. (2001). *Treponema parvum* sp. nov., a small, glucuronic or galacturonic acid-dependent oral spirochaete from lesions of human periodontitis and acute necrotizing ulcerative gingivitis. *Int J Syst Evol Microbiol* **51(Pt3)**, 955-962.

Wyss, C., Moter, A., Choi, B.K., Dewhirst, F.E., Xue, Y., Schüpbach, P., Göbel, U.B., Paster, B.J. & 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(Pt4)**, 1117-1122.

Zinicola, M., Lima, F., Lima, S., Machado, V., Gomez, M., Döpfer, D., Guard, C. & Bicalho, R. (2015). Altered microbiomes in bovine digital dermatitis lesions, and the gut as a pathogen reservoir. *PLoS ONE* **10(3)**, e0120504.

Ziolecki, A. (1979). Isolation and characterization of large treponemes from the bovine rumen. *Appl Environ Microbiol* **37(1)**, 131-135.

Ziolecki, A. & Wojciechowicz, M. (1980). Small pectinolytic spirochetes from the rumen. *Appl Environ Microbiol* **39(4)**, 919-922.

Tables

Table 1. An enzyme activity profile comparison between the bovine GI tract isolate (Ru1) and other related bovine, porcine and human treponemes as determined by the API® ZYM system.

Enzymes tested: 1, alkaline phosphatase; 2, C4 esterase; 3, C8 esterase lipase; 4, C14 lipase; 5, leucine arylamidase; 6, valine arylamidase; 7, cystine arylamidase; 8, trypsin; 9, chymotrypsin; 10, acid phosphatase; 11, naphtholphohydrolase; 12, α-galactosidase; 13, β-galactosidase; 14, β-glucuronidase; 15, α-glucosidase; 16, β-glucosidase; 17, N-acetyl-β-glucosaminidase; 18, α-mannosidase; 19, α-fucosidase. +, positive; -, negative.

***Treponema* strain**	**Presence of enzyme activity**																		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
Treponema ruminis Ru1^{T‡}	-	-	+	-	+	-	-	-	-	-	-	-	+	-	-	+	-	-	-
Treponema porcinum ATCC BAA-908^{T†}	-	+	-	-	-	-	-	-	-	+	+	-	-	-	+	-	-	-	-
Treponema berlinense ATCC BAA-909^{T†}	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-
Treponema pedis DSM 18691^{T*}	-	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
Treponema brennaborense CIP 105900^{T§}	+	+	+	-	-	-	-	-	-	+	+	-	+	-	+	-	+	-	-
Treponema pectinovorum ATCC 33768$^{T\#}$	-	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-
Treponema socranskii subsp. *socranskii* ATCC 35536$^{T\#}$	+	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-
Treponema maltophilum ATCC 51939$^{T\#}$	+	+	+	-	-	-	-	-	-	+	+	+	-	-	+	-	-	-	+
Treponema amylovorum ATCC 700288$^{T\|}$	+	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	+
Treponema medium ATCC 700293^{T*}	+	+	+	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Treponema putidum ATCC 700334^{T+}	+	+	+	-	+	-	-	+	+	+	+	+	+	-	+	+	-	-	-
Treponema denticola ATCC 35405^{T+}	-	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
Treponema parvum ATCC 700770$^{T\alpha}$	+	+	+	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-
Treponema lecithinolyticum OMZ 684$^{T\beta}$	+	+	+	-	-	-	-	-	-	+	+	-	+	+	-	-	+	-	+

API® ZYM profile reported by [‡]Evans *et al.*, 2011, [†]Nordhoff *et al.*, 2005, [*]Evans *et al.*, 2009, [§]Schrank *et al.*, 1999, [#]Wyss *et al.*, 1996, [|]Wyss *et al.*, 1997, [+]Wyss *et al.*, 2004, [α]Wyss *et al.*, 2001, [β]Wyss *et al.*, 1999.

Figure Legends

Fig. 1. A phylogenetic tree of maximum-likelihood illustrating 16S rRNA gene sequence comparisons over 1,312 aligned bases between the bovine GI tract isolate (Ru1) and all other recognised *Treponema* spp.. Bootstrap confidence intervals, based on 10,000 reiterations, are shown as percentages at the nodes; values below 40% were removed for clarity. Genbank accession numbers are given in parentheses next to each strain. Bar, 0.02 nucleotide substitutions per site.

Fig. 2. A phylogenetic tree of maximum-likelihood illustrating the gene sequence comparisons across 479 aligned bases encoding recombinase A (*recA*) between the bovine GI tract isolate (Ru1) and all available sequences from other recognised *Treponema* spp.. Bootstrap confidence intervals, based on 10,000 reiterations, are shown as percentages at the nodes; values below 40% were removed for clarity. Genbank accession numbers are given in parentheses next to each strain. Bar, 0.05 nucleotide substitutions per site.