Treponema rectale sp. nov., a spirochete isolated from the bovine rectum

Gareth J. Staton,¹* Kerry Newbrook,¹ Simon R. Clegg,¹ Richard J. Birtles,² Nicholas J. Evans¹ and Stuart D. Carter¹

Abstract

A Gram-stain-negative, obligate anaerobic spirochete, CHPA¹, was isolated from the rectal tissue of a Holstein–Friesian cow. On the basis of 16S rRNA gene comparisons, CHPA¹ was most closely related to the human oral spirochete, Treponema parvum, with 88.8% sequence identity. Further characterisation on the basis of recA gene sequence analysis, cell morphology, pattern of growth and physiological profiling identified marked differences with respect to other recognised species of the genus Treponema. Microscopically, the helical cells measured approximately 1–5 µm long and 0.15–0.25 µm wide, with two to five irregular spirals. Transmission electron microscopy identified four periplasmic flagella in a 2:4:2 arrangement. CHPA¹ grew independently of serum, demonstrated no evidence of haemolytic activity and possessed an in vitro enzyme activity profile that is unique amongst validly named species of the genus Treponema, exhibiting C4 esterase, α-galactosidase and β-galactosidase activity. Taken together, these data indicate that CHPA¹ represents a novel species of the genus Treponema, for which the name Treponema rectale is proposed. The type strain of Treponema rectale is CHPA¹ (=DSM 103679®=NCTC 13848®).

Species of the genus Treponema are typically anaerobic, fastidious and highly motile microorganisms with a spiral morphology that are capable of occupying a diverse range of hosts and tissues, including the oral cavity and genital tract of humans, the gastrointestinal (GI) tract and feet of ruminants, and the digestive tracts of insects [1-4]. Whereas GI colonisation has been associated with commensalism, several taxa have been shown to play a pathogenic role in a number of diseases, including bovine digital dermatitis [5], periodontal disease [6] and syphilis [7]. To date, there are 28 species of the genus Treponema with validly published names, with one of these, Treponema socranskii, having been delineated into three subspecies [8].

The mammalian GI tract harbours a complex symbiotic community of microorganisms, numerous in both abundance and diversity. Spirochetes are known to be a common inhabitant of the GI tract and occur at relatively high densities in healthy animals, including in the rumen of cattle [9, 10]. However, despite early confirmation of the presence of a large number of morphologically and physiologically diverse spirochete species in the bovine rumen [11], their fastidious nature has, for the most part, hindered their characterisation. Moreover, little is known about the spirochetes present in other regions of the bovine GI tract.

Species of the genus Treponema, in particular, are thought to comprise a significant, yet poorly understood, proportion of the spirochetes that reside within the bovine GI tract. The bovine rumen harbours several phylotypes within the genus Treponema, three taxa of which have been classified to date: Treponema bryantii [12], Treponema saccharophilum [13] and Treponema ruminis [14]. As part of an investigation into the microbial diversity of the bovine GI tract, Evans et al. [15] used 16S rRNA gene sequence comparisons to delineate bovine GI tract treponeme isolates into four novel phylotypes. Since all four novel phylotypes shared less than 97% sequence identity with established members of the genus Treponema, it is suggested that, on the basis of current taxonomic criteria [16], they may each represent a novel species. In the present study, these findings have been combined with new genotypic and phenotypic data to support the proposal that one of these phylotypes (phylotype 2; CHPA¹), represents a novel species of the genus Treponema.

CHPA¹ was recovered from a post-mortem rectal tissue biopsy collected from a single Holstein–Friesian cow in Cheshire, United Kingdom, immediately after slaughter, as described previously [15]. CHPA¹ was maintained in the laboratory by passage every 24 h in Oral Treponeme Enrichment Broth (OTEB; Anaerobe Systems) supplemented with...
10% (v/v) rabbit serum (RS; GE Healthcare Life Sciences), under anaerobic conditions (N₂/H₂/CO₂, 85:10:5, 36°C). Phase contrast microscopy confirmed the presence of helically-coiled spirochete cells in the liquid media that displayed high levels of rotational and translational motility when observed in wet mounts. Cultured treponemes were stored at -80°C in growth medium containing 10% (v/v) glycerol and were revived successfully. The bacterial morphology of this strain was examined by transmission electron microscopy and has been reported previously [17]. Cells, when grown in OTEB, were observed to be approximately 1–5 μm long and 0.15–0.25 μm wide. CHPAᵀ exhibited typical spirochaetal helical morphology, with two to five irregular spirals and four periplasmic flagella, originating at the poles and overlapping centrally, to yield a 2:4:2 arrangement.

Bacteria also grew when sub-cultured onto unsupplemented fastidious anaerobe agar (FAA) plates (LabM), forming singular circular, convex, punctiform colonies of approximately 0.2 mm in diameter after 10 days of incubation. Inoculation onto FAA plates that did not contain serum failed to retard growth, and cells were thereafter successfully sub-cultured in OTEB without serum supplementation, indicating that these treponemes were serum-independent under the conditions of in vitro culture [15]. Colonies were observed to be translucent, lacked a metallic sheen and there was no evidence of local β-haemolysis. These colonies

**Fig. 1.** A molecular phylogenetic analysis of 16S rRNA sequences from all currently recognised species of the genus *Treponema*, inferred using the maximum-likelihood method based on the Tamura–Nei model, from gene sequence comparisons across 1309 aligned bases. Accession numbers are shown in parentheses. Bootstrap values, based on 10,000 iterations, are shown as percentages at the nodes. Bar, 0.05 nucleotide substitutions per site.
differed markedly in both size and appearance from those formed by other treponemes of the GI tract, including the spherical, opaque colonies of *Treponema saccharophilum* ATCC 43261\textsuperscript{T} and *Treponema succinifaciens* ATCC 33096\textsuperscript{T}, with reported colony diameters of 3–4 mm [13] of 4–8 mm [18] respectively, and the irregular, greyish colonies of *Treponema berlinense* ATCC BAA-909\textsuperscript{T}, with a reported colony size of 1–2 mm in diameter [19]. The colonies of *Treponema ruminis* DSM 103462\textsuperscript{T}, although similar in appearance, varied somewhat in size (0.2–0.5 mm) [14], an observation not made for CHPA\textsuperscript{T} (0.2 mm). It is noted however that the extent to which variable culture conditions contribute to these differences remains undefined.

Genomic DNA preparation, 16S rRNA gene PCR amplification and sequencing were performed as described previously by Evans et al. [15]. Sequencing of this amplification product yielded 1309 base pairs (bp) of unambiguous sequence data (GenBank accession number GU566699). A comparison of this sequence with the 16S rRNA sequences available in GenBank confirmed it to be most similar to the 16S rRNA gene sequences of the members of the genus *Treponema*. A 1309 bp 16S rRNA gene sequence alignment of CHPA\textsuperscript{T} and members of the genus *Treponema* was generated using CLUSTALW [20] and trimmed in the BioEdit sequence alignment editor [21]. The 16S rRNA gene of CHPA shared 84.4 and 88.0\% sequence similarity with the two other previously identified bovine GI tract treponemes, *T. saccharophilum* ATCC 43261\textsuperscript{T} and *Treponema bryantii* ATCC 33254\textsuperscript{T}, respectively. In sharing 88.8\% sequence identity, CHPA\textsuperscript{T} is most closely related to *Treponema parvum* ATCC 700770\textsuperscript{T}, a spirochete isolated from the human oral cavity that has been implicated in periodontal disease [22]. From a 16S rRNA gene sequence alignment of all valid treponemal species, phylogeny was inferred using the maximum-likelihood method with nucleotide substitution rates calculated according to the Tamura–Nei model [23] in MEGA 6 [24], selected as the best-fit evolutionary model using TOPALi 2.5 [25]. The robustness of the proposed tree branching was evaluated using bootstrap analysis (10 000 iterations).

In the proposed tree (Fig. 1), the phylogenetic distance between CHPA\textsuperscript{T} and its nearest neighbour was at least that observed between several validly named species of the genus *Treponema*. CHPA\textsuperscript{T} was observed to cluster with a number of commensal species of the genus *Treponema* isolated from, or associated with, the GI tract of several mammalian hosts: *T. bryantii* ATCC 33254\textsuperscript{T}, isolated from the bovine rumen [12], *T. succinifaciens* ATCC 33096\textsuperscript{T}, isolated from *Treponema parvum* ATCC 700770\textsuperscript{T}, a spirochete isolated from human oral cavity.

![Fig. 2. A molecular phylogenetic analysis of available recombinase A (recA) sequences from members of the genus *Treponema* inferred using the maximum-likelihood method based on the Kimura two-parameter model, from gene sequence comparisons across 293 aligned bases. Accession numbers are shown in parentheses. Bootstrap values, based on 10 000 iterations, are shown as percentages at the nodes. Bar, 0.05 nucleotide substitutions per site.](attachment:image.png)
the porcine colon [18] and *Treponema porcinum* ATCC BAA-908T, isolated from porcine faeces [19], sharing 88.0, 85.4 and 88.7 % 16S rRNA gene sequence identity, respectively. Phylogenetic reconstruction placed CHPA1 within a deep-rooted clade that is occupied by the aforementioned commensal treponemes as well as a number of oral species of the genus *Treponema*, including the most closely related known relative of CHPA1, *T. parvum* ATCC 700770T.

The phylogenetic position of CHPA within the genus *Treponema* was further explored using inferences derived from an alignment of recombinase A gene (recA) sequences. Degenerate primers suitable for the amplification of a recA fragment were used as described previously [14]. The PCR primers (recA forward 5'-GCAACYTTGTTCTTTACR-3' and recA reverse 5'-GAAATGTACGGTCCYGAA-3') and template DNA were added to a *Taq* polymerase master mix, prepared according to manufacturer's instructions (Qiagen). Temperature cycling consisted of an initial denaturation of 95 °C for 6 min, followed by 40 cycles of 95 °C for 15 s, 48.2 °C for 15 s and 72 °C for 1 min, followed by a final extension of 72 °C for 7 min. Sequencing of the amplification product yielded 455 bp of unambiguous sequence data. Sequencing results were viewed and edited using ChromasPro 2.0.0. (Technelysium), and submitted to Genbank (accession number KX501214). This 455 bp fragment was then aligned against the recA genes of relevant characterised species of the genus *Treponema* using CLUSTALW [20] using sequences trimmed in the BioEdit sequence alignment editor [21]. TOPALi 2.5 [25] was utilised to identify the best-fit evolutionary model for phylogenetic reconstruction. Phylogeny was subsequently inferred using the Kimura two-parameter model [26] using MEGA6 [24]. The robustness of the proposed tree branching was evaluated using bootstrap analysis (10 000 iterations).

In contrast to the relatively high (>80 %) 16S rRNA sequence homology observed across a diverse range of species of the genus *Treponema*, recA gene sequence homology between CHPA1 and this selection of organisms was generally lower, ranging from 67.6 to 82.5 %. Comparison of these data with recA sequences available from species of the genus *Treponema* revealed that CHPA1 shared the highest recA sequence similarity with *T. succinifaciens* ATCC 33096T (82.5 %). Phylogenetic inference, performed on the available recA sequences of members of the genus *Treponema* as described above, resulted in CHPA1 being loosely clustered with the GI tract treponemes *T. saccharophilum* ATCC 43261T, *T. succinifaciens* ATCC 33096T and *T. ruminis* DSM 103462T (Fig. 2).

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**Table 1.** An enzyme activity profile comparison between the bovine GI tract isolate CHPA1 and other related bovine, porcine and human treponemes as determined using the API ZYM system.

<table>
<thead>
<tr>
<th>Species/subspecies</th>
<th>Strain</th>
<th>Presence of enzyme activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td><em>Treponema rectale</em></td>
<td>CHPA1T</td>
<td>–</td>
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<tr>
<td><em>Treponema ruminis</em></td>
<td>DSM 103462T</td>
<td>–</td>
</tr>
<tr>
<td><em>Treponema parvum</em></td>
<td>ATCC 700770T</td>
<td>+</td>
</tr>
<tr>
<td><em>Treponema berinense</em></td>
<td>ATCC BAA-909T</td>
<td>–</td>
</tr>
<tr>
<td><em>Treponema porcinum</em></td>
<td>ATCC BAA-908T</td>
<td>–</td>
</tr>
<tr>
<td><em>Treponema pedis</em></td>
<td>DSM 18691T</td>
<td>–</td>
</tr>
<tr>
<td><em>Treponema medium</em></td>
<td>ATCC 700293T</td>
<td>+</td>
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<tr>
<td><em>Treponema brennaborense</em></td>
<td>DSM 12168T</td>
<td>+</td>
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<tr>
<td><em>Treponema pectinovorum</em></td>
<td>ATCC 33768T</td>
<td>–</td>
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<tr>
<td><em>Treponema socranskii subsp. socranski</em></td>
<td>ATCC 35536T</td>
<td>+</td>
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<tr>
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<td>ATCC 35334T</td>
<td>+</td>
</tr>
<tr>
<td><em>Treponema socranskii subsp. paredi</em></td>
<td>ATCC 35335T</td>
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<td><em>Treponema multophilia</em></td>
<td>ATCC 51939T</td>
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<td><em>Treponema amylovorum</em></td>
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<td><em>Treponema denticola</em></td>
<td>ATCC 35405T</td>
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<tr>
<td><em>Treponema putidum</em></td>
<td>ATCC 700334T</td>
<td>+</td>
</tr>
<tr>
<td><em>Treponema lecithovorum</em></td>
<td>ATCC 700332T</td>
<td>+</td>
</tr>
</tbody>
</table>

*API ZYM data previously reported by *Evans et al* [15], *Wyss et al* [22], *Nordhoff et al* [19], *Evans et al* [27], *Schrank et al* [28], *Wyss et al* [29], *Wyss et al* [30], *Wyss et al* [31], *Wyss et al* [32].
The type strain, CHPA, was isolated from the rectal tissue of a Holstein–Friesian cow from a dairy farm in Cheshire, UK.

DESCRIPTION OF TREPONEMA RECTALE SP. NOV.

Trepotema rectale (rec.ta'le. N.L. neut. adj. rectale, pertaining to the rectum, rectal, referring to the source of isolation).

Cells are small Gram-stain-negative, obligate anaerobic spirochetes, indigenous to the bovine GI tract. Under phase contrast microscopy, cells are identified as highly motile spirochete cells with a helical coil. Cells measure approximately 1–5 µm long, 0.15–0.25 µm wide, with two to five irregular spirals. Transmission electron microscopy identifies four periplasmic flagella, in a 2:4:2 arrangement. Cells require a 24 h anaerobic incubation at 36 °C to reach stationary phase in OTEB. Cells do not require serum supplementation to grow. In culture, rotational and translational movement is evident; cells exhibit jerky flexing. When streaked onto FAA plates with or without 10 % RS, colonies grow to approximately 0.2 mm in diameter after 10 days. There is no evidence of β-haemolysis after three weeks' incubation. API ZYM analysis identifies positive reactions for C4 esterase, α-galactosidase and β-galactosidase and negative reactions for alkaline phosphatase, C8 esterase lipase, C14 lipase, leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, acid phosphatase, naphtholphosphohydrolase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. The type strain, CHPA (=DSM 103679=NTCC 13848) was isolated from the rectal tissue of a Holstein–Friesian cow from a dairy farm in Cheshire, UK.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
All sampling undertaken was approved by the University of Liverpool Ethical Review Process under approved ethics application number VREC137.

References


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