The genus *Prevotella* has been reclassified from *Bacteroides* in 1990, and *Prevotella melaninogenica* is the type species (Shah & Collins, 1990). To date, the number of species within the family Prevotellaceae has risen to 51 (Euzeby 1997). Species of the genus *Prevotella* are part of the human oral and gastrointestinal microbiome. Infections from members of the genus *Prevotella* arise especially when translocation occurs into primarily sterile body sites, presenting as either polymicrobial or monomicrobial infections. Various sites of the body can be affected, such as the oral cavity (e.g. acute dental abscess), the central nervous system (e.g. brain abscess), as well as the skin (soft tissue abscess) (Le Moal et al., 2003; Mehmood et al., 2014; Robertson & Smith, 2009).

This study describes the phenotypical, biochemical and genotypical characterization of strain A1336<sup>T</sup>, concluding that it represents a novel species. Comparative 16S rRNA gene sequence analysis was used to determine the phylogenetic relationships between the strain and other members of the family Prevotellaceae.

The strain was isolated from the wound of a 77 year old female patient, as part of a mixed infection involving aerobic and other anaerobic bacteria, namely *Enterococcus faecalis*, *Staphylococcus haemolyticus*, a species of the genus *Corynebacterium*, *Fusobacterium nucleatum* and *Porphyromonas somerae*. Primary cultivation was on brain–heart infusion (BHI) agar (Oxoid) with addition of 5% defibrinated sheep blood (Acila) and supplemented with IsoVitalex enrichment (BD) under anaerobic conditions (5% H<sub>2</sub> and 10% CO<sub>2</sub> in N<sub>2</sub>) at 37 °C. The strain also grew on various solid and liquid enriched media (chocolate agar, supplemented Columbia sheep blood agar, Schäldner agar, liver broth and CMC broth) and exhibited moderate haemolysis on blood-containing solid media. The strain did not grow in the presence of oxygen and should therefore be considered obligately anaerobic. Motility was not observed. The strain exhibited a temperature optimum for growth of 35–37 °C and tolerated 30 °C, but did not grow at 45 °C. The optimal pH for growth was 7–9 with reduced growth at pH 6 and no growth at pH 5 on BHI agar with sheep blood. Colony morphologies appeared as circular, convex, shiny, weakly pigmented with a greyish-brown color as examined on supplemented BHI with sheep blood or on Columbia agar after incubation for 2–5 days, and developed toffee brown pigmentation after a prolonged incubation of 10 days, which was best visible on Schäldner agar.

Light microscopy after Gram staining and transmission electron microscopy (TEM) were used to investigate the cell morphology. Cells were Gram-stain-negative, appeared without spores and presented as pleomorphic short rods, measuring 0.4–0.6 × 0.9–1.3 µm, with a few cells as long as 2.5 µm. In TEM ultra-thin sections, vesicles could be visualized outside the cells (Fig. 1a, b). There were also thin, tube-like structures extending from the cells, possibly
allowing for interconnectivity between cells (Fig. 1b). Similar structures have been described previously in species of the genus *Prevotella* (Hedberg et al., 2013).

The nucleotide sequence of the 16S rRNA gene of strain A1336<sup>T</sup> was determined as described previously (Carlier et al., 2004). For comparative phylogenetic and sequence similarity analysis, 16S rRNA gene sequences from all species in the family Prevotellaceae were retrieved from the EzTaxon database (Kim et al., 2012). Phylogenetic analysis was based on full-length 16S rRNA and showed the strain A1336<sup>T</sup> to represent a member of the genus *Prevotella*, but to be different from the other species of the genus *Prevotella* and *Hallella seregens* (Fig. 2). In the maximum likelihood phylogenetic tree reconstructed from aligned sequences, *Prevotella bergensis* and *Prevotella multisaccharivorax* were the most closely related to the novel strain. Compared with strain A1336<sup>T</sup>, *P. bergensis* also had the highest 16S rRNA gene sequence similarity (91.54%), as calculated by Clustal Omega (McWilliam et al., 2013). The range of 16S sequence similarities of strain A1336<sup>T</sup> with all currently named species of the genus *Prevotella* were in the range of 91.54%–86.78% with the exception of *Prevotella heparinolytica* (82.83%) and *Prevotella zoogloeoformans* (82.08%). These latter two 16S sequence similarities are approximately as low as those for similarity to members of the genus *Alloprevotella*

<sup>sp.</sup> (82.22–82.94%). The genus *Alloprevotella* has recently been proposed to accommodate the former *Prevotella tumnerae* together with the newly described species *Alloprevotella rava* (Downes et al., 2013). Phylogenetically, *P. heparinolytica* and *P. zoogloeoformans* sequences appear more distinct from the 16S rRNA gene sequences of other species of the genus *Prevotella* and the Subcommittee on the Taxonomy of Gram-Negative Anaerobic Rods recommends that these two species be designated *Bacteroides heparinolyticus* and *Bacteroides zoogloeoformans*. However, they have not yet been reclassified and are still formally considered species of the genus *Prevotella* (Olsen & Shah, 2001). In contrast to this, *Hallella seregens* has been classified as a member of the genus *Prevotella* but no formal proposal has been made to change the genus name (Willems & Collins, 1995). For completeness, species in the genera *Prevotella*, *Hallella*, *Paraprevotella* and *Alloprevotella* are all included in the phylogenetic tree depicting the family Prevotellaceae (Fig. 2).

The G+C content of genomic DNA was 43.2 mol%, as determined by high-performance liquid chromatography (HPLC) at the Identification Service of the DSMZ, Braunschweig, Germany. This value lies within the range reported for the species in the genus *Prevotella* (39–60 mol%) (Avgustin, 1997, Willems & Collins, 1995). Compared with the phylogenetically most closely related species, strain A1336<sup>T</sup> can clearly be distinguished by its genomic DNA G+C content from *P. bergensis* [48 mol%; (Downes et al., 2006)] and *P. multisaccharivorax* [49.9 mol%; (Sakamoto et al., 2005)].

Cellular fatty acid (CFA) methyl ester analysis was carried out by the Identification Service of the DSMZ, Braunschweig, Germany, after the strain had been grown anaerobically on supplemented BHI agar with sheep blood at 37 °C.
Fig. 2. Phylogenetic tree showing strain A1336<sup>T</sup> in relation to all 51 currently described species of the family Prevotellaceae based on 16S rRNA gene sequences. Species of the genera *Alloprevotella* and *Paraprevotella* are included as outgroups and *Alloprevotella tannerae* is presented as the root of the tree. Branch support was based on 2000 bootstrap replicates. Numbers represent bootstrap values for each branch. Accession numbers for 16S rRNA gene sequences are given in brackets for each strain. Branch lengths are measured as the number of substitutions per site as indicated by the bar.
and harvested after 48 h. Identification and quantification of the CFA by gas chromatography (GC) is based on the MIDI Microbial Identification System (MIDI). The results are shown in Table S1 (available in the online Supplementary Material). The cellular long-chain fatty acid profile predominantly comprises 3-OH iso-C\textsubscript{17:0} (15.5 %), anteiso-C\textsubscript{15:0} (15.2 %) and iso-C\textsubscript{15:0} (12.4 %).

The analysis of biochemical metabolic characteristics showed that the strain exhibits both moderately saccharolytic as well as proteolytic activity (Table S2). Strain A1336\textsuperscript{T} gave positive reactions in the rapid ID 32A panel (bioMérieux) for β-galactosidase, α-glucosidase, β-N-acetylglucosaminidase, alkaline phosphatase, arginine arylamidase, leucyl glycine arylamidase, alanine arylamidase and glutamyl glutamic acid arylamidase. Reactions for β-galactosidase 6-phosphate, β-glucosidase, α-arabinosidase and raffinose fermentation were very weak and considered negative. Negative reactions were also obtained for the remaining 20 reactions, resulting in profile 0501 4502 0 0. All reactions were carried out in triplicate. Strain A1336\textsuperscript{T} was positive for fermentation of cellobiose, glucose, lactose, maltose, mannose, raffinose and sucrose, whereas no acid was produced from arabinose, mannitol, melezitose, salicin, sorbitol, trehalose and xylose. Sugar fermentation was measured using peptone–yeast extract broth (adjusted to pH 8.0) containing the respective sugars and read out by pH change (below 5.5, positive, and above 6.0, negative, respectively). All reactions were carried out in triplicate. In the rapid ID 32A, however, fermentation reactions for mannose and raffinose were negative, which may be due to a limitation of miniaturized biochemical panels. P. bergensis as the phylogenetically closest relative was tested for sugar fermentation in parallel to strain A1336\textsuperscript{T}, and there were differences for fermentation of arabinose, raffinose, salicin, sucrose and xylose, which can be used for differentiation. Catalase reaction (15 % H\textsubscript{2}O\textsubscript{2}) and indole production were both negative. An overview of phenotypic characteristics is provided in Table 1, comparing strain A1336\textsuperscript{T} with the phylogenetically most closely related species and the type species of the genus (P. melaninogenica).

Diagnostic susceptibility testing of strain A1336\textsuperscript{T} showed no zone of inhibition for colistin (10 µg) and vancomycin (5 µg), performed by disk diffusion on supplemented BHI agar with sheep blood (both BD) under anaerobic conditions. The strain proved to be susceptible to 10 % bile (Ox bile dried pure, Merck) performed by disk diffusion on supplemented BHI agar with sheep blood under anaerobic conditions, a well known characteristic which distinguishes species of the genus Prevotella from species of the genus Bacteroides (Shah & Collins, 1990). A1336\textsuperscript{T} was highly susceptible to clinically relevant antibiotics. Using the E-Test method, amoxicillin-clavulate (bioMérieux) had minimal inhibitory concentrations (MICs) below 0.016, as tested on Brucella agar (BD) with 5 % defibrinated sheep blood and vitamin K1 supplementation under anaerobic conditions. Clindamycin (Liofilchem) and penicillin G (Liofilchem) had both MICs of 0.016. Meropenem (Liofilchem) and metronidazole (Liofilchem) had MICs of 0.023 and 0.125, respectively. Vancomycin resistance was confirmed by E-Test (Liofilchem) with a MIC of 6.

We conclude that strain A1336\textsuperscript{T} represents a novel species of the genus Prevotella, for which the name Prevotella colorans sp. nov. is proposed.

**Description of Prevotella colorans sp. nov.**

*Prevotella colorans* [coˈlo.rans] (L. part. adj. colorans coloring, referring to the slow production of greyish-brown pigment upon incubation on solid media).

Cells are obligately anaerobic, Gram-stain-negative, non-motile, pleomorphic short rods (0.4–0.6×0.9–1.3 µm,

<table>
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<tr>
<th>Characteristic</th>
<th>1</th>
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<th>3</th>
<th>4</th>
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<tbody>
<tr>
<td>Pigment production</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
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<td>Fermentation reactions:</td>
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<td>Arabinose</td>
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<td>Cellobiose</td>
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<td>Raffinose</td>
<td>+</td>
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<td>Salicin</td>
<td>−</td>
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<td>Sucrose</td>
<td>+</td>
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</tr>
<tr>
<td>Xylose</td>
<td>−</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Genomic G+C content of DNA (mol%)</td>
<td>43.2</td>
<td>48.0</td>
<td>49.9</td>
<td>41.1</td>
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</table>

Table 1. Differential phenotypic characteristics of *Prevotella* A1336 sp. nov. and related species of the family Prevotellaceae

Strains: 1, A1336; 2, *P. bergensis* DSM 17361; 3, *P. multisaccharivorax* PPPA20(T) =JCM 12954(T) =DSM 17128(T) (Sakamoto et al., 2005); 4, *P. melaninogenica* (type species) ATCC 25845 =Finegold B282 =VPI 4196 (Downes et al., 2006; Sakamoto et al., 2005) +, positive; −, negative; v, variable.
with a few cells up to 2.5 µm long). After 2–5 days of incubation on rich media such as supplemented BHI agar with sheep blood, colonies are approximately 1 mm in diameter, circular, convex, shiny and weakly pigmented with a greyish-brown color. Upon further incubation, colonies develop a toffee brown pigmentation, in contrast to the phylogenetically most closely related species which have not been described as producing pigment. Anaerobic growth occurs at 37, 36, 35 and 30 °C, but not at 45 °C. The optimum pH range is 7–9. Cells are moderately saccharolytic and proteolytic. Cells are able to ferment cellobiose, glucose, lactose, maltose, mannose, raffinose and sucrose, but acid is not lytic. The type strain is A1336 (=DSM 100333 =CCUG 67421), isolated from a human wound. The genomic DNA G+C content of the type strain is 43.2 mol%.

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References:


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