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Sutterella stercoricanis sp. nov., isolated from canine faeces

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Morphological, biochemical and molecular genetic studies were carried out on an unknown non-spore-forming, Gram-negative, rod-shaped bacterium which was isolated from dog faeces. The bacterium grew under anaerobic conditions, was asaccharolytic, resistant to 20 % (v/v) bile and was oxidase- and urease-negative. Phylogenetic analysis based on comparative 16S rRNA gene sequencing showed that the unidentified bacterium clustered with *Sutterella wadsworthensis*, although a sequence divergence of >5 % indicated that the bacterium from dog faeces represented a previously unrecognized subline within the genus. On the basis of the presented findings, a novel species, *Sutterella stercoricanis* sp. nov., is described. The type strain of *Sutterella stercoricanis* is 5BAC4^T (=CCUG 47620^T=CIP 108024^T).

The genus *Sutterella* was proposed by Wexler *et al.* (1996) to accommodate some *Campylobacter gracilis*-like organisms from human clinical sources. The organisms consisted of bile-resistant, asaccharolytic, nitrate-positive, urease-negative, Gram-negative rods that were phenotypically and genotypically distinct from *Campylobacter* (formerly *Bacteroides*) *gracilis*. Currently, only a single species of the genus *Sutterella* is known, namely *Sutterella wadsworthensis* (Wexler *et al.*, 1996). The genus *Sutterella* belongs in the family *Alcaligenaceae* within the *β-Proteobacteria*. To date, *S. wadsworthensis* has been isolated from patients with a variety of gastrointestinal infections (Wexler *et al.*, 1996), and there is evidence (Engberg *et al.*, 2000) that it occurs in human faeces. During the course of a study on taxonomically problematic organisms within the canine gut, we have characterized an unusual Gram-negative, short, rod-shaped organism from faeces which phenotypically resembles the genus *Sutterella*. In this article, we report the phenotypic characteristics of this *Sutterella*-like organism and the results of a phylogenetic study. On the basis of the presented findings, we propose that the unknown organism be designated a novel species of the genus *Sutterella*, for which the name *Sutterella stercoricanis* sp. nov. is proposed.

Strain 5BAC4^T was isolated from the faeces of a healthy male Labrador dog. Faecal samples were collected immediately following defecation and used to prepare a 10 % (w/v) slurry using pre-reduced 0·1 M PBS (pH 7). The slurry was transferred into an anaerobic cabinet (10 % H_2, 10 % CO_2, 80 % N_2) and homogenized for 10 min. Serial 10-fold dilutions were prepared using half-strength peptone water and cysteine hydrochloride (0·5 g l^{-1}). Strain 5BAC4^T was isolated from bacteroides agar (Holdeman *et al.*, 1977) that had been incubated at 37 °C for 48 h, from a 10^{-5} dilution. The organism was isolated from two out of five faecal samples examined (counts of 4·8 and 5·7 $\log_{10}$ c.f.u. g^{-1}). Haemolysis was tested on anaerobic blood agar (Oxoid). Cellular morphology and motility were examined in peptone/yeast extract/glucose broth and from anaerobic blood agar. Aerotolerance was determined by incubating the organism on brucella agar plates with and without 5 % (v/v) laked sheep blood in an atmosphere containing either 2 or 6 % oxygen, as described by Wexler *et al.* (1996). Traditional biochemical tests were performed as described by Holdeman *et al.* (1977). The oxidase test was performed as described by Summanen *et al.* (1993). Urease activity was determined using urease discs (Rosco Diagnostics). Biochemical tests were also carried out using the commercially available API Rapid ID 32A and API ZYM systems (bioMérieux) according to the manufacturer's instructions. Long-chain cellular fatty acids were analysed by the MIDI system (Microbial ID). For the determination of G+C content, DNA was isolated after disruption of cells using a French pressure cell and then purified in hydroxyapatite. The G+C content (mol%) was determined by using HPLC after digestion of DNA with P1 nuclease and alkaline phosphatase, as described by Mesbah *et al.* (1989). DNA–DNA

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CCUG 47620^T is AJ566849.

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reassociation experiments were carried out according to the spectrophotometric method of De Ley *et al.* (1970), with the modification described by Huß *et al.* (1983) and Escara & Hutton (1980) using a Gilford System model 2600 spectrometer equipped with a Gilford model 2527-R thermoprogrammer and plotter. The 16S rRNA genes of the isolate were amplified by a PCR using universal primers pA (positions 8–28, *Escherichia coli* numbering) and pH* (positions 1542–1522). The amplified product was purified using a QIAquick PCR purification kit (Qiagen) and directly sequenced using primers directed towards conserved positions of the rRNA gene, the dRhodamine terminator cycle sequencing kit (PE Applied Biosystems) and an automatic DNA sequencer (model 377; PE Applied Biosystems). The closest known relatives of the new isolate were determined by performing database searches using the program FASTA (Pearson & Lipman, 1985). Closely related sequences were retrieved from EMBL and aligned with the newly determined sequence using the program SEQtools (Rasmussen, 2002). The resulting multiple sequence alignment had approximately 100 bases at the 5′ end of the rRNA omitted from further analysis, because of alignment uncertainties due to the highly variable region V1, using the program GeneDoc (Nicholas *et al.*, 1997). A phylogenetic tree was reconstructed according to the neighbour-joining method with the programs SEQtools and TREEVIEW (Page, 1996) and the stability of the groupings was estimated by bootstrap analysis (1000 replications) using the same programs.

The faecal isolate consisted of non-spore-forming, short, rod-shaped cells. Typical cells were 0·8 μm wide and 1·5–2·0 μm in length. The organism grew under anaerobic conditions and in the presence of 2 % oxygen, but not in oxygen at 6 % or more. It was catalase- and oxidase-negative and asaccharolytic in traditional and API test systems. In the commercially available API Rapid ID 32A and API ZYM test systems, the unidentified isolate was very unreactive; apart from a weak activity for glycine arylamidase all other tests were negative. The long-chain cellular fatty acids consisted of 3-OH $C_{12:0}$ (2·2 %), iso-2-OH $C_{13:0}$, (0·6 %), $C_{14:0}$ (9·1 %), iso-$C_{14:0}$ (2·2 %), iso-$C_{15:0}$ (1·3 %), $C_{16:0}$ (42·1 %), $C_{16:1}$*cis*9 (16·7 %), $C_{18:0}$ (6·1 %), $C_{18:1}$*cis*9 (13·6 %) and $C_{18:1}$*cis*11 (6·4 %). To ascertain the phylogenetic position of the unknown organism, comparative 16S rRNA gene sequencing was carried out. The almost complete sequence (>1400 nt) of the 16S rRNA gene of the isolate was determined. Sequence database searches revealed highest sequence relatedness to *S. wadsworthensis* (94·7 % sequence similarity); other taxa (e.g. *Achromobacter*, *Alcaligenes* and *Bordetella* species, *Leptothrix discophora*) were more distantly related (data not shown). Treeing analysis confirmed that the unknown dog-gut bacterium was phylogenetically most closely associated with *S. wadsworthensis* (Fig. 1). DNA–DNA reassociation was conducted between the unknown strain, 5BAC4^T, and *S. wadsworthensis* CCUG 42229^T. The two strains displayed 35 % DNA relatedness, consistent with separate species status.

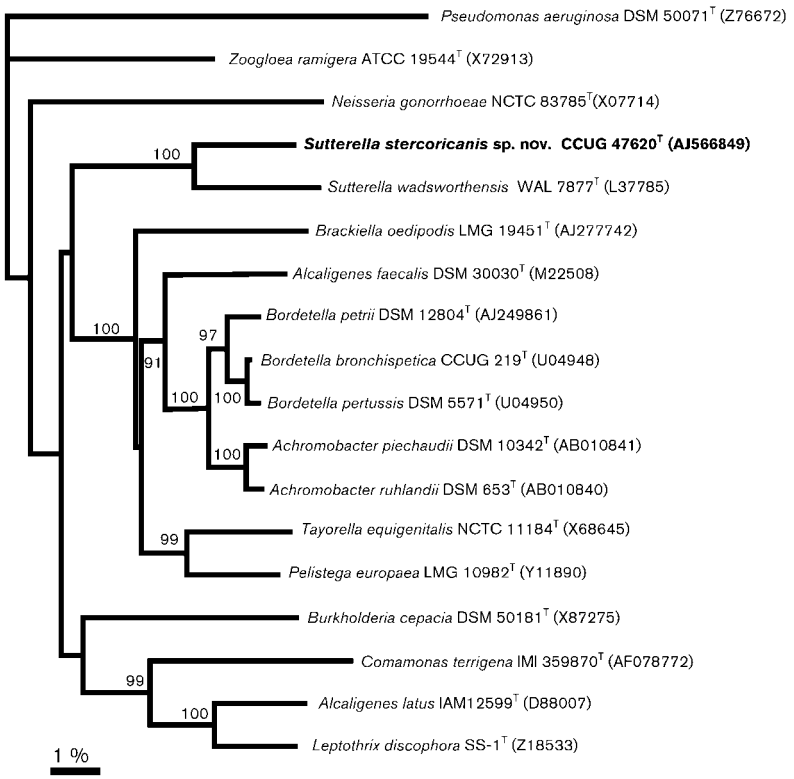

Fig. 1. Unrooted tree showing the phylogenetic relationships of *S. stercoricanis* sp. nov. and its close relatives. The tree, constructed using the neighbour-joining method, was based on a comparison of approximately 1300 nt. Bootstrap values expressed as a percentage of 1000 replications are given at the branching points.

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It is evident from the results of the taxonomic study that the Gram-negative, rod-shaped isolate recovered from dog faeces represents a hitherto unknown species. Phylogenetically, the novel organism displayed a statistically highly significant association with *S. wadsworthensis*, the clustering together of these organisms being supported by a bootstrap resampling value of 100 % (Fig. 1). The affinity between the dog bacterium and *S. wadsworthensis* was strongly supported in terms of phenotypic criteria. In particular, the dog bacterium closely resembled the genus *Sutterella* in being asaccharolytic, oxidase-negative, urease-negative, bile-resistant and nitrate-positive. Further support for the close association between the unidentified organism and *S. wadsworthensis* was evident from long-chain cellular fatty acid data, the major fatty acids being $C_{14:0}$, $C_{16:0}$, $C_{16:1}$*cis*9 and $C_{18:1}$*cis*9.

Although clustering of the unknown dog bacterium with *S. wadsworthensis* was statistically significant, a sequence divergence of >5 % between these taxa indicated that the relationship was not particularly close. While there is no precise cut-off for delineating species based on sequence divergence values alone, it is universally recognized that divergence values of 3 % or more indicate that organisms are not related at the species level (Stackebrandt & Goebel, 1994). The observed 16S rRNA gene divergence value between the unknown dog bacterium and *S. wadsworthensis* clearly demonstrates that these organisms represent closely related, albeit different, genetic species. This result was confirmed by DNA–DNA reassociation which demonstrated that the novel isolate and *S. wadsworthensis* represent different genospecies. Therefore, on the basis of the presented findings we propose that the unknown rod-shaped organism from dog faeces be assigned to the genus *Sutterella* as a novel species, *Sutterella stercoricanis* sp. nov. *S. stercoricanis* phenotypically closely resembles *S. wadsworthensis*, but can be distinguished from the latter by its negative leucine arylamidase, arginine arylamidase and alkaline phosphatase reactions in the API Rapid ID 32A test system. Although only a single strain of *S. stercoricanis* is currently known, we consider that the formal description of this species, together with 16S rRNA gene sequence data and biochemical criteria to aid its identification, will facilitate its recognition in the routine laboratory, thereby permitting the recovery of additional strains.

Description of *Sutterella stercoricanis* sp. nov.

Sutterella stercoricanis (ster.co.ri.ca′nis. L. n. *stercus*, *-oris* faeces; L. gen. n. *canis* of the dog; N.L. gen. n. *stercoricanis* from dog faeces).

Cells consist of short, straight rods, approximately $0{\cdot}8 \times 1{\cdot}5$–2 μm, which stain Gram-negative. No spores are observed. Non-haemolytic. Grows under anaerobic and microaerophilic conditions, growing in 2 %, but not 6 %, oxygen. Resistant to 20 % (v/v) bile. Oxidase- and catalase-negative. Nitrate is reduced. Asaccharolytic. Using the commercially available API test systems, no activity is detected for acid phosphatase, alkaline phosphatase, alanine arylamidase, α-arabinosidase, arginine arylamidase, arginine dihydrolase, esterase C4, ester lipase C8, α-chymotrypsin, cystine arylamidase, α-fucosidase, α-glucosidase, β-glucosidase, β-glucuronidase, α-galactosidase, β-galactosidase, β-galactosidase-6-phosphate, glutamic acid decarboxylase, glutamyl glutamic acid arylamidase, histidine arylamidase, leucine arylamidase, leucyl glycine arylamidase, lipase C14, α-mannosidase, *N*-acetyl-β-glucosaminidase, proline arylamidase, phenylalanine arylamidase, phosphoamidase, pyroglutamic acid arylamidase, serine arylamidase, trypsin, valine arylamidase, urease or tyrosine arylamidase. Weak activity for glycine arylamidase may be detected. Indole is not produced. The long-chain cellular fatty acids are mainly of the straight-chain saturated and monounsaturated types, with $C_{14:0}$, $C_{16:0}$, $C_{16:1}$ and $C_{18:1}$ predominating.

The type strain is 5BAC4^T (=CCUG 47620^T=CIP 108024^T). The G+C content of DNA of the type strain is 60 mol%. Isolated from faeces of a healthy male Labrador dog.

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