Dysgonomonas gen. nov. to accommodate
Dysgonomonas gadei sp. nov., an organism
isolated from a human gall bladder, and
Dysgonomonas capnocytophagoides
(formerly CDC group DF-3)

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Results of a polyphasic taxonomic study on an unknown Gram-negative,
facultatively anaerobic, coccobacillus-shaped organism isolated from an
infected human gall bladder are presented. Phenotypic and molecular
taxonomic studies revealed the organism to be close to, but distinct from,
organisms designated CDC (Centers for Disease Control and Prevention) group
DF-3. The unknown bacterium was readily distinguished from reference strains
of Bacteroides, Prevotella, Porphyromonas and related taxa by 16S rRNA gene
sequencing, biochemical tests, analysis of cellular long-chain fatty acids and
electrophoretic analysis of whole-cell proteins. Based on the results of the
present study, it is proposed that the unknown bacterium be classified in a
new genus, Dysgonomonas, as Dysgonomonas gadei sp. nov. (type strain CCUG
42882T = CIP 106420T). In addition, a new species, Dysgonomonas
capnocytophagoides sp. nov., is proposed to accommodate strains previously
belonging to CDC group DF-3. The type species of the genus Dysgonomonas is
Dysgonomonas gadei.

Keywords: taxonomy, phylogeny, Dysgonomonas, CDC group DF-3, 16S rRNA

INTRODUCTION

CDC (Centers for Disease Control and Prevention) group DF-3 (dysgonic fermenter 3) is a group of
hitherto unclassified, fastidious, facultatively anaerobic, Gram-negative coccobacilli (Wallace et al.,
1989). CDC group DF-3 strains grow slowly on blood agar medium, but not on MacConkey agar, ferment
several carbohydrates, hydrolyse aesculin, do not reduce nitrate, and are catalase- and oxidase-negative.
These organisms have a distinct cellular fatty acid profile characterized by large amounts of straight-
chain saturated, anteiso- and iso-methyl branched, and 3-hydroxy long-chain fatty acids (Wallace et al.,
1989). Comparative 16S rRNA sequence analysis has indicated that CDC group DF-3 is phylogenetically
related to, but nevertheless distinct from Bacteroides, Porphyromonas, Prevotella and related species (Paster et
al., 1994; Vandamme et al., 1996). CDC DF-3 isolates are resistant to several antimicrobial agents,
including penicillins, cephalosporins, aminoglycosides and ciprofloxacin. Most isolates are susceptible to
clindamycin, tetracycline, chloramphenicol, imipenem and trimethoprim/sulfamethoxazole (Gill et al., 1991;
Blum et al., 1992). CDC group DF-3 organisms are relatively rare isolates that have been recovered from
stool samples, mainly in immunocompromised patients and patients with severe underlying diseases
(Blum et al., 1992; Heiner et al., 1992), but also from clinical materials such as blood, wounds and abscesses
(Aronson & Zbick, 1988; Bangsborg et al., 1990). The pathogenic potential of the organisms remains un-
known. The isolation of organisms with biochemical properties similar, but not identical, to those of CDC
group DF-3 (designated CDC group DF-3-like
organisms) has also been reported (Daneshvar et al., 1991).

In this study, the cultural and biochemical properties, cellular fatty acid composition and SDS-PAGE protein profile of an isolate, which resembles CDC group DF-3 organisms, recovered from an infected gall bladder are reported. In addition, results of 16S rRNA gene sequencing indicate that the organism represents a new species that clusters together with CDC group DF-3 organisms. It is therefore proposed that a new genus, Dysgonomonas, be created to accommodate the new organism isolated from an infected gall bladder classified as Dysgonomonas gadei sp. nov. (type strain CCUG 42882T = CIP 106420T) and organisms previously designated CDC group DF-3 be classified as Dysgonomonas capnocytophagoides sp. nov. The type species of the genus Dysgonomonas is Dysgonomonas gadei.

METHODS

Source of the organism. Strain CCUG 42882T was originally isolated from a 68-year-old male with known non-insulin-dependent diabetes mellitus and essential hypertension. The patient was admitted to hospital because of fractures of the left tibia and the left third, fourth and fifth metatarsal bones following a car accident. Treatment was conservative. Nine days later, the patient was suddenly ill with fever, chills and vascular collapse. Escherichia coli was recovered in blood cultures. Ultrasound scanning of the abdomen, performed a few days later, showed gallstones and a distended gall bladder. Aerobic and anaerobic cultivation of pus (which contained no trace of bile) aspirated from the gall bladder yielded growth of E. coli, a Klebsiella species and enterococci. In addition, a slow-growing, Gram-negative coccobacillus was recovered by anaerobic cultivation on kanamycin/ vancomycin laked blood agar. The patient was treated with cefuroxime and metronidazole for 2 weeks and recovered uneventfully.

Cultural and biochemical characterization. The unknown Gram-negative coccobacillus from the infected gall bladder was cultured aerobically at 35 °C in a thermostat supplemented with 7–5% CO₂ on Fastidious Anaerobic Agar (FAA; Lab M) and Columbia agar base (Difco), both of which were supplemented with 5% human blood. Growth requirements for X and V factors were examined using discs impregnated with X factor, V factor or both, and nutrient agar as basal medium. Bile sensitivity was determined by the Oxgall test using Diatabs diagnostic tablets (Rosco) as described by Weinberg et al. (1983). The strain was biochemically characterized by using a combination of conventional tests and the API ID32A, API ID32E and API ZYM systems according to the manufacturer’s instructions (API bioMérieux).

Susceptibility to antimicrobial agents. MICs of a range of antibacterial agents, including penicillins, cephalosporins, glycopeptide antibiotics, aminoglycosides, fluoroquinolones, macrolides, quinolones, chloramphenicol, doxycycline, metronidazole, sulphaadine and trimethoprim/sulphamethoxazole, were examined using the E-test (AB Biodisk). The tests were performed as recommended by the manufacturers and read after incubation aerobically for 2 d. MIC of metronidazole was determined after 2 d anaerobic incubation (Mart Anoxomat system; Mart Microbiology Automation). FAA, supplemented with 5% human blood, was used as growth medium instead of PDM Antibiotic Sensitivity medium because of poor growth on the latter, even when supplemented with 5% horse blood.

SDS-PAGE of whole-cell proteins. To assess the overall phenotypic resemblance of the new isolate and reference species, a comparative analysis of whole-cell protein profiles by SDS-PAGE was performed. PAGE analysis of whole-cell proteins was performed as described by Pot et al. (1994) and Vandamme et al. (1998). For densitometric analysis, normalization and interpretation of protein patterns the GCW 3.0 software package (Applied Maths) was used. The similarity between all pairs of traces was expressed by the Pearson product–moment correlation coefficient, converted for convenience to a percentage similarity.

Long-chain cellular fatty acid analysis. Cells were cultured on chocolate agar using Columbia agar base and incubated for 48 h at 37 °C and centrifuged. Saponification, methanolysis, extraction and identification of the fatty acid methyl esters were made using the Microbial Identification system (Microbial ID) as described previously (Moore et al., 1994; Debelen et al., 1997).

Determination of 16S rRNA gene sequences and phylogenetic analysis. Phylogenetic determination was performed by comparative 16S rRNA gene sequence analyses. A large fragment of the 16S rRNA gene (corresponding to positions 30–1521 of the E. coli 16S rRNA gene) was amplified by PCR using conserved primers close to the 3' and 5' ends of the gene. The PCR products were directly sequenced using a Taq dye-deoxy terminator cycle sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model 373A; Applied Biosystems). The closest known relatives of the new isolate were determined by performing a database search using the program FASTA of the Genetics Computer Group package (Pearson & Lipman, 1985). These sequences and those of other known related strains were retrieved from the EMBL or Ribosomal Database Project databases and aligned with the newly determined sequences using the program FILEUP (Devereux et al., 1984). The resulting multiple sequence alignment was corrected manually and approximately 100 bases at the 5' end of the rRNA were omitted from further analyses because of alignment ambiguities. Pairwise evolutionary distances were then computed from a continuous stretch of 1320 bases using the correction of Jukes & Cantor (1969). A phylogenetic tree was constructed according to the neighbour-joining method with the program NEIGHBOR (Felsenstein, 1989). The stability of the groupings was estimated by bootstrap analysis (500 replications) using the programs SEQBOOT, DNADIST, NEIGHBOR and CONSENSE (Felsenstein, 1989).

RESULTS

Cultural and biochemical properties

The isolate originating from the infected gall bladder consisted of non-motile, Gram-negative coccobacilli that grew relatively slowly on blood agar. The organism grew with or without the addition of 7.5% CO₂, in addition to growing anaerobically. After 48 h incubation aerobically at 35 °C in a CO₂-enriched atmosphere (7.5%), the colonies were 1–2 mm in
Dysgonomonas gen. nov.

The unknown clinical isolate was sensitive to metronidazole (MIC 1.5 µg ml⁻¹), clindamycin (MIC 0.25 µg ml⁻¹), doxycycline (MIC 0.19 µg ml⁻¹), imipenem (MIC 0.5 µg ml⁻¹), meropenem (MIC 0.064 µg ml⁻¹) and trimethoprim/sulphamethoxazole (MIC 0.125 µg ml⁻¹). The organism was resistant to cefoxitin (MIC 24 µg ml⁻¹) and the other cephalosporins tested [cefotaxime, cefpirome, ceftazidime, ceftriaxone, cefuroxime and cephalothin (MIC 256 µg ml⁻¹)]. In addition, the isolate was resistant to the aminoglycosides [gentamicin (MIC 256 µg ml⁻¹), netilmicin (MIC 256 µg ml⁻¹) and sulphadiazine (MIC 256 µg ml⁻¹)], fluoroquinolones [ciprofloxacin (MIC 32 µg ml⁻¹) and oxafloxacin (MIC 8 µg ml⁻¹)] and the
glycopeptide antibiotics [vancomycin (MIC 48 µg ml⁻¹) and teicoplanin (MIC 256 µg ml⁻¹)]. The MICs of other antibiotics tested were: ampicillin, 6 µg ml⁻¹; amoxicillin, 6 µg ml⁻¹; amoxicillin/clavulanic acid, 1 µg ml⁻¹; benzylpenicillin, > 32 µg ml⁻¹; erythromycin, 16 µg ml⁻¹; azithromycin, 16 µg ml⁻¹; clarithromycin, 16 µg ml⁻¹; and chloramphenicol, 4 µg ml⁻¹.

**Phylogenetic analysis**
To investigate the genealogical affinity between the unknown bacterium and its relationship with other Gram-negative taxa, comparative 16S rRNA gene sequence analysis was performed. The almost complete gene sequence (> 1400 nt) of the unknown isolate was determined. Sequence searches of GenBank and RDP databases revealed that the unknown isolate was phylogenetically most closely associated with members of the genera *Bacteroides*, *Prevotella* and *Porphyromonas*, which belong to the *Bacteroides* subgroup of the flavobacterium–bacteroides subphyllum. A tree constructed by the neighbour-joining method depicting the phylogenetic affinity of the unknown coccobacillus is shown in Fig. 2. The unknown bacterium was phylogenetically placed on the periphery of members of the genus *Porphyromonas* clustering with CDC group DF-3 strain CCUG 17996.

**Table 1.** Composition (%) of cellular fatty acids in strain CCUG 42882T and CDC group DF-3 strains CCUG 17996T and 42515

<table>
<thead>
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<th>Fatty acid</th>
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<td></td>
<td>DF-3 CCUG 17996T</td>
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ND, Not detected.
Dysgonomonas gen. nov.

Fig. 2. Unrooted tree showing the phylogenetic relationships of Dysgonomonas species and some related Gram-negative bacteria. The tree constructed using the neighbour-joining method was based on a comparison of approx. 1327 nt. Bootstrap values, expressed as a percentage of 500 replications, are given at branching points. Scale bar, 1% sequence divergence.

A second strain belonging to CDC group DF-3 (CCUG 42515) was found to be identical to CCUG 17996 (100% sequence similarity in 1420 bases compared). The sequence divergence between strain CCUG 42882T and CDC group DF-3 strains was 7%.

DISCUSSION

It is evident from the findings of this investigation that the bacterium originating from a human gall bladder infection represents a hitherto unrecognized species within the Bacteroides subgroup of the flavobacterium–bacteroides subphylum. Phylogenetically, the coccobacillus-shaped bacterium clustered together with strain CDC group DF-3 (93% sequence similarity) and bootstrap resampling showed this relationship to be statistically significant (100% recovery in 500 resamplings). Other taxa displayed significantly lower levels of sequence similarity including Bacteroides (85–87%), Porphyromonas (84–88%) and Prevotella (79–85%) and the misclassified strict anaerobes Bacteroides distasonis (86% sequence similarity), Bacteroides forsythus (89% sequence similarity), Bacteroides merdae (89% sequence similarity) and Bacteroides splanchnicus (83% sequence similarity) (Fig. 2). Based on tree topology considerations and sequence divergence values, it is clear that the unknown bacterium cannot be assigned to any of the currently described genera. The association between the unknown bacterium and the CDC group DF-3 strains is, however, very significant and the depth of the cluster formed by the two taxa is indicative of a single genus. Chemotaxonomic findings (analysis of whole-cell proteins and long-chain fatty acids) and its facultative nature also demonstrate the separateness of the unknown clinical isolate from currently named members of the Bacteroides subgroup and strongly support its affinity with the CDC group DF-3 strains, which also exhibit a facultative mode of respiration. Until a more complete inventory of the organisms present within this supercluster of bacteria is established, it is not possible to draw conclusions on the significance of the association of facultative organisms with strict anaerobes. The recovery of the newly
Dysgonomonas capnocytophagoides

Dysgonomonas capnocytophagoides is described as a Gram-negative, coccolid, non-motile, non-sporulating organism. It is facultatively anaerobic, with growth observed in a CO₂-enriched atmosphere. The colonies are 1–2 mm in diameter, non-adherent, grey-white, and smooth. It is oxidase- and catalase-negative, and produces acid from glucose but no gas. The organism grows at 25 °C but not at 43 °C. It does not reduce nitrate or produce hydrogen sulphide or acetoin. Aesculin is hydrolysed but gelatin and urea are not. Indole is produced. It is resistant to ox bile. Glucose is fermented with production of acid but no gas.

Dysgonomonas capnocytophagoides is different from Prevotella and Porphyromonas species, as it does not produce hydrogen sulphide or acetoin. It grows on MacConkey agar and produces acid from glucose but no gas. It is resistant to ox bile. Glucose is fermented with production of acid but no gas.

Dysgonomonas capnocytophagoides is an opportunistic pathogen, often found in the oral cavity, where it is associated with periodontal disease. It has been isolated from human clinical specimens and stool samples.

The description of Dysgonomonas capnocytophagoides is based on results obtained in this study and those obtained by Wallace et al. (1989). The cells are non-motile, and the organism grows at 35 °C in a CO₂-enriched atmosphere. The colonies are 1–2 mm in diameter, non-adherent, grey-white, and smooth. The organism does not reduce nitrate or produce hydrogen sulphide or acetoin. Aesculin is hydrolysed but gelatin and urea are not. Indole is produced. It is resistant to ox bile.

The organism is not reduced by nitrogen and does not produce hydrogen sulphide or acetoin. Aesculin may or may not be hydrolysed but gelatin and urea are not. Indole may or may not be produced. The organism is not reduced by nitrate and produces hydrogen sulphide or acetoin. Aesculin is hydrolysed but gelatin and urea are not. Indole may or may not be produced.
fermentation are propionic, lactic and succinic acids. Glucose is fermented with production of acid but no gas. Acid is produced from L-arabinose, lactose, maltose, D-mannose, melibiose, raffinose, sucrose and D-xylose. Adonitol, D-arabitol, L-arabitol, dulcitol, inositol, D-mannitol, D-sorbitol and trehalose are not acidified. Positive reactions are obtained for acid phosphatase, alanine arylamidase, alkaline phosphatase, α-arabinosidase, α-galactosidase, β-galactosidase, β-galactosidase 6-phosphate, α-glucosidase, β-glucosidase, glutamyl glutamic acid arylamidase, leucyl glycine arylamidase and phosphomimidase. Arginine arylamidase, arginine dihydrolase, N-acetyl-β-glucosaminidase, α-fucosidase, β-glucuronidase, glutamic acid decarboxylase, glycine arylamidase, histidine arylamidase, lysine decarboxylase, leucine arylamidase, α-mannosidase, ornithine decarboxylase, chymotrypsin, cystine arylamidase, ester lipase C8, lipase C14, phenylalanine arylamidase, proline arylamidase, pyrogallotannic acid arylamidase, serine arylamidase, tyrosine arylamidase, trypsin and valine arylamidase are not detected. Esterase C4 production is variable. Isolated from human clinical specimens. Habitat is not known. The type strain is CCUG 17996T (= LMG 11519T). The G + C content of DNA of the type strain is 38 mol %.

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REFERENCES


