***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 42882^T = CIP 106420^T). 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; Bangsberg *et al.*, 1990). The pathogenic potential of the organisms remains unknown. The isolation of organisms with biochemical properties similar, but not identical, to those of CDC group DF-3 (designated CDC group DF-3-like

Abbreviation: CDC, Centers for Disease Control and Prevention.

The GenBank accession number for the 16S rRNA gene sequence of *Dysgonomonas gadei* strain CCUG 42882^T is Y18530.

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 42882^T = CIP 106420^T) 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 42882^T 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, sulphadiazine 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; Debelian *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 PILEUP (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

Fig. 1. Similarity dendrogram based on whole-cell protein patterns of *Dysgonomonas* gen. nov., sp. nov. and related species. Levels of correlation are expressed as percentages of similarity for convenience.

diameter, non-adherent, entire, grey-white, smooth and non-haemolytic, and had a slightly aromatic odour. Following incubation for a few more days, the colonies were somewhat coalesced, butyrous and α -haemolytic. Growth was obtained at 25 °C, but not at 43 °C. Growth was not observed on MacConkey agar. The coccobacillus grew on nutrient agar around X and XV discs, suggesting a growth factor dependency for haem. The organism grew under microaerophilic and strictly anaerobic conditions. It was catalase-positive and oxidase-negative, it failed to reduce nitrate, and it did not produce hydrogen sulphide or acetoin, but did produce indole. Aesculin was hydrolysed, but gelatin and urea were not. Growth and precipitation were observed around the Oxgall tablets demonstrating that the organism was resistant to ox bile. Glucose was fermented with production of acid but no gas. Acid was produced from L-arabinose, cellobiose, fructose, lactose, D-mannose, raffinose, L-rhamnose, D-ribose (weak reaction), salicin, starch, sucrose and trehalose. Adonitol, dulcitol, erythritol, glycogen, inositol, D-mannitol and D-sorbitol were not acidified. Positive reactions were obtained for *N*-acetyl- β -glucosaminidase, acid phosphatase, alanine arylamidase, alkaline phosphatase, α -arabinosidase, ester lipase C8 (weak reaction), α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, β -glucuronidase, glutamyl glutamic acid arylamidase, α -mannosidase (weak reaction), α -fucosidase, chymotrypsin, alanine arylamidase, leucyl glycine arylamidase, phosphoamidase and trypsin. Arginine dihydrolase, arginine arylamidase, cystine arylamidase, esterase C-4, β -galactosidase 6-phosphate, glutamic acid decarboxylase, glycine arylamidase, histidine arylamidase, lipase C14, leucine arylamidase, phenylalanine arylamidase, proline arylamidase, pyroglutamic acid arylamidase, serine arylamidase, tyrosine arylamidase, urease and valine arylamidase were not detected. Results for the production of β -glucuronidase differed between the

different API systems employed. Using the API ID32A and rapid ID32E systems, β -glucuronidase was detected, but with the API ZYM kit this enzyme was not produced. The API rapid ID32A profile for the unknown bacterium was 4577640222 when compared to 4736440202 obtained for DF-3 strains CCUG 17996^T and CCUG 42515.

SDS-PAGE of whole-cell proteins

A numerical analysis of the whole-cell protein patterns of the unknown bacterium and two CDC group DF-3 strains (CCUG 17996, CCUG 42515) together with reference strains of *Bacteroides*, *Capnocytophaga* and *Prevotella* is shown in Fig. 1. The unknown gall bladder isolate was shown to be separate from the two DF-3 strains and all reference strains used. The nearest correlation was with *Bacteroides uniformis* at a level of approximately 58%. In addition, the two CDC DF-3 strains clustered together with a correlation level of about 75%.

Susceptibility to antimicrobial agents

The unknown clinical isolate was sensitive to metronidazole (MIC 1.5 $\mu\text{g ml}^{-1}$), clindamycin (MIC 0.25 $\mu\text{g ml}^{-1}$), doxycycline (MIC 0.19 $\mu\text{g ml}^{-1}$), imipenem (MIC 0.5 $\mu\text{g ml}^{-1}$), meropenem (MIC 0.064 $\mu\text{g ml}^{-1}$) and trimethoprim/sulphamethoxazole (MIC 0.125 $\mu\text{g ml}^{-1}$). The organism was resistant to cefoxitin (MIC 24 $\mu\text{g ml}^{-1}$) and the other cephalosporins tested [cefotaxime, cefpirome, ceftazidime, ceftriaxone, cefuroxime and cephalothin (MIC 256 $\mu\text{g ml}^{-1}$)]. In addition, the isolate was resistant to the aminoglycosides [gentamicin (MIC 256 $\mu\text{g ml}^{-1}$), netilmicin (MIC 256 $\mu\text{g ml}^{-1}$) and sulphadiazine (MIC 256 $\mu\text{g ml}^{-1}$)], fluoroquinolones [ciprofloxacin (MIC 32 $\mu\text{g ml}^{-1}$) and oxafloxacin (MIC 8 $\mu\text{g ml}^{-1}$)] and the

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

ND, Not detected.

Fatty acid	Strain		
	DF-3 CCUG 17996 ^T	DF-3 CCUG 42515	CCUG 42882 ^T
iso-C _{12:0}	ND	0.3	1.9
C _{13:0}	ND	0.9	ND
iso-C _{13:0}	9.5	0.5	0.9
anteiso-C _{13:0}	ND	0.7	3.5
C _{14:0}	2.4	1.9	1.6
iso-C _{14:0}	19.8	12.3	12.9
C _{15:0}	11.3	14.2	2.9
C _{15:1}	3.4	2.7	ND
anteiso-C _{15:0}	19.6	19.4	23.9
iso-C _{15:0}	2.7	2.2	4.0
iso-3OH C _{15:0}	ND	0.8	ND
3OH C _{15:0}	ND	3.8	ND
3OH C _{16:0}	4.6	5.3	7.9
iso-3OH C _{17:0}	3.3	3.5	ND
C _{16:0}	3.4	3.6	15.2
iso-C _{16:0}	ND	ND	1.3
iso-3OH C _{16:0}	12.3	9.3	10.5
3OH C _{16:0}	4.6	5.3	7.9
C _{16:0 cis9}	4.7	3.9	1.2
iso-3OH C _{17:0}	3.3	3.5	ND
3OH C _{17:0}	ND	1.6	0.5
anteiso-C _{17:1}	3.2	ND	ND
2OH C _{17:0}	ND	ND	1.8
C _{18:0 cis9}	ND	1.4	1.1

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.

Cellular fatty acid composition

The percentage fatty acid compositions of strain CCUG 42882^T and CDC group DF-3 strains (CCUG 17996 and CCUG 42515) are given in Table 1. The fatty acids of the unknown isolate CCUG 42882^T were found to be iso-C_{14:0}, anteiso-C_{15:0}, C_{16:0} and iso-3OH C_{16:0}. This fatty acid pattern was similar to those of the two reference CDC group DF-3 strains examined.

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 subphylum. 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.

described organism from the diseased gall bladder may be indicative that it was present as an opportunistic pathogen. Moore *et al.* (1994) found that anteiso- $C_{15:0}$, iso- $C_{15:0}$, iso-3OH $C_{17:0}$ and $C_{16:0}$ are the major cellular fatty acids in *Bacteroides* and *Prevotella*. These findings were consistent with earlier studies (Miyagawa *et al.*, 1979; Shah & Collins 1980; Mayberry *et al.*, 1982). There is a general consensus that iso- $C_{15:0}$ is the major fatty acid in *Porphyromonas* strains, accounting for 33–58% of the fatty acids present (Moore *et al.*, 1994). Therefore, the fatty acid profile of strain CCUG 42882^T is incompatible with the genus *Porphyromonas*. The fatty acids of the aforementioned strain, however, closely resemble those reported for CDC group DF-3 strains (Daneshvar *et al.*, 1991; Wallace *et al.*, 1989; Table 1). Therefore, based on both phenotypic and phylogenetic findings of this and previous studies, it is proposed that the unknown Gram-negative coccobacillus and organisms previously designated CDC group DF-3 merit classification in a new genus, *Dysgonomonas* gen. nov., as *Dysgonomonas gadei* sp. nov. and *Dysgonomonas capnocytophagoides* sp. nov., respectively.

Description of *Dysgonomonas* gen. nov.

Dysgonomonas (Dys.go.no.mo'nas. N.L. n. *Dysgonic* a type of fermenter; Gr. fem. n. *monas* a monad, unit; N.L. fem. n. *Dysgonomonas* a monad from a *Dysgonic* fermenter).

Cells consist of non-motile, Gram-negative coccobacilli to short rods. Growth is not observed on MacConkey agar. Requires X growth factor. Facultatively anaerobic. May be catalase-positive or -negative. Oxidase-negative. Glucose is fermented producing acid but no gas. Alkaline phosphatase is produced but not arginine dihydrolase. Does not reduce nitrate. Hydrogen sulphide and acetoin are not produced. Aesculin may or may not be hydrolysed but gelatin and urea are not. Indole may or may not be produced. The long-chain cellular fatty acids are of the straight-chain saturated, anteiso- and iso-methyl branched and 3-hydroxy types. The G+C content of DNA is 38 mol% (Vandamme *et al.*, 1996). Isolated from human clinical specimens and stool samples. Habitat is not known. The type species is *Dysgonomonas gadei*.

Description of *Dysgonomonas gadei* sp. nov.

Dysgonomonas gadei (ga'de.i. N.L. gen. masc. n. *gadei* of Gade Institute, Bergen, Norway where the organism was first isolated).

Cells consist of non-motile, Gram-negative coccobacilli that grow relatively slowly on blood agar. After 48 h incubation aerobically at 35 °C in a CO₂-enriched atmosphere, the colonies are 1–2 mm in diameter, non-adherent, entire, grey-white, smooth and non-haemolytic, and have a slightly aromatic odour. Following incubation for a few more days, the colonies become

somewhat coalesced, butyrous and α -haemolytic. Growth is obtained at 25 °C but not at 43 °C. Grows under microaerophilic and strictly anaerobic conditions. The organism does not grow on MacConkey agar but grows on nutrient agar around X and XV discs, suggesting a growth factor dependency for haem. The organism is catalase-positive and oxidase-negative. It does not reduce nitrate or produce hydrogen sulphide or acetoin. Aesculin is hydrolysed but gelatin and urea are not. Indole is produced. Resistant to ox bile. Glucose is fermented with production of acid but no gas. Acid is produced from L-arabinose, cellobiose, fructose, lactose, D-mannose, melezitose, melibiose, raffinose, L-rhamnose, D-ribose (weak reaction), salicin, starch, sucrose, trehalose and xylose. Adonitol, dulcitol, erythritol, glycogen, inositol, D-mannitol and D-sorbitol are not acidified. Positive reactions for *N*-acetyl- β -glucosaminidase, acid phosphatase, alanine arylamidase, alkaline phosphatase, α -arabinosidase, ester lipase C8 (weak reaction), α -galactosidase, β -galactosidase (weak reaction), α -glucosidase, β -glucosidase, glutamyl glutamic acid arylamidase, α -mannosidase (weak reaction), α -fucosidase, chymotrypsin, alanine arylamidase, leucyl glycine arylamidase, phosphoamidase and trypsin. Arginine dihydrolase, arginine arylamidase, cystine arylamidase, esterase C-4, β -galactosidase 6-phosphate, glutamic acid decarboxylase, glycine arylamidase, histidine arylamidase, lipase C14, leucine arylamidase, phenylalanine arylamidase, proline arylamidase, pyroglutamic acid arylamidase, serine arylamidase, tyrosine arylamidase, urease and valine arylamidase are not detected. The type strain of *Dysgonomonas gadei* is CCUG 42882^T (= CIP 106420^T). The type strain was recovered from a human infected gall bladder. Habitat is not known.

Description of *Dysgonomonas capnocytophagoides* sp. nov.

Dysgonomonas capnocytophagoides (cap.no.cy.to.pha.goi'des. Gr. n. *kapnos* smoke; Gr. n. *kytos* hollow vessel; Gr. v. *phagein* to eat; Gr. adj. suffix *-oides* alike; N.L. adj. *capnocytophagoides* capnocytophagalike, referring to some properties shared between these organisms).

The description is based on results obtained in this study and those obtained by Wallace *et al.* (1989). Cells consist of non-motile, Gram-negative coccobacilli to short rods. After 48 h incubation aerobically at 35 °C in a CO₂-enriched atmosphere (7.5%), the colonies are 1–2 mm in diameter, non-adherent, entire, grey-white, smooth and non-haemolytic and have a slight aromatic odour. The organism does not grow on MacConkey agar. Facultatively anaerobic. The organism is catalase- and oxidase-negative. It 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. Resistant to ox bile. Nitrate is not reduced. Major products of glucose

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 phosphoamidase. 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, pyroglutamic acid arylamidase, serine arylamidase, tyrosine arylamidase, trypsin and valine arylamidase are not detected. Esterase C-4 production is variable. Isolated from human clinical specimens. Habitat is not known. The type strain is CCUG 17996^T (= LMG 11519^T). The G + C content of DNA of the type strain is 38 mol %.

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