Megamonas rupellensis sp. nov., an anaerobe isolated from the caecum of a duck

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We report here the identification, characterization and culture of a Gram-negative to Gram-variable, rod-shaped, non-spore-forming anaerobic bacterium (strain FM1025T) isolated from the caecum of a duck. Phylogenetic analysis based on comparative 16S rRNA gene sequencing showed that this strain clustered with species of the family ‘Acidaminococcaceae’, with 94.9 % similarity to Megamonas hypermegala DSM 1672T and less than 91 % similarity with type strains of Pectinatus species. Sequence similarities of at least 98–99 % were observed with numerous sequences deposited in GenBank of uncultured strains from human and chicken caecal contents, but this strain is the first isolate of this taxon to be cultivated and described. On the basis of morphological, physiological and phylogenetic features, this strain should be assigned to a novel species in the genus Megamonas, for which the name Megamonas rupellensis sp. nov. is proposed. The type strain is strain FM1025T (=DSM 19944T =CIP 109788T).

The microbial composition of the chicken intestine evolves with age and varies in its different sections. It is estimated that 60–90 % of the intestinal bacterial community can not currently be cultivated (Lu et al., 2003). The dominant micro-organisms in the gut include bacteria related to Clostridium, Lactobacillus, Bacteroides and Megamonas (Lan et al., 2002; Zhu et al., 2002). This complex microflora is known to play a key role in the health of birds. For instance, young chickens are very sensitive to enteropathogen infections because their intestinal micro-organisms are not fully established (Nurmi & Rantala, 1973). In 2000, searching for bacteria that produce anti-enteropathogen molecules, Portrait et al. (2000) isolated an anaerobic strain (FM1025T) from the caecum of a duck. This strain was preliminarily identified as Fusobacterium mortiferum using API galleries. In the present work, we have characterized this bacterium further and, using 16S rRNA gene sequence analysis, we have shown that strain FM1025T represents a novel species belonging to the genus Megamonas (Shah & Collins, 1982).

Strain FM1025T was grown routinely at 37 °C on TGY broth (w/v; 3 % tryptone, 2 % yeast extract, 0.5 % glucose, 0.05 % l-cysteine hydrochloride) using either anaerobic jars (AnaeroGen; Oxoid) or an anaerobic chamber containing 10 % H₂, 10 % CO₂ and 80 % N₂ (MACS DG500; Don Whitley Scientific). Strain FM1025T was restreaked weekly onto TGY agar [TGY broth solidified with 1.2 % (w/v) agar] and could be stored for several years after mixing 0.5 ml of an overnight culture with 0.5 ml 30 % (w/v) glycerol. Unless otherwise specified, growth of strain FM1025T was tested in 250 ml TGY broth incubated at 37 °C under continuous stirring (160 r.p.m.) in the anaerobic chamber.

Strain FM1025T grew optimally at 37 °C and the pH range for growth was pH 5–9 with an optimum at pH 7. On solid M63 minimal medium (Miller, 1972) supplemented with the reducing amino acid l-cysteine hydrochloride (0.05 % w/v), no colonies were observed after 72 h of incubation. In the same medium enriched with 0.2 % yeast extract, colonies could be visualized after 48 h of incubation. Strain FM1025T was anaerobic, non-motile and oxidase- and catalase-negative.

After growth on solid medium or in liquid broth for 12, 24 or 48 h, cultures were Gram-negative to Gram-variable. The strain was unsporulated in TGY but intracellular inclusions could be seen on Gram stain preparations. For electron microscopy, 5 ml aliquots of a 48 h culture were centrifuged for 3 min at 5000 g. Cell pellets were washed twice with 50 mM sodium cacodylate buffer (pH 7) and prepared as described by Dongard & Bordes (1991). The preparations were examined using a Philips Quanta 200 ESEM/FEG microscope with an Everhart Thornley-BSE detector. Cells were rod-shaped (1.0–6.0 μm) and occurred singly, in pairs and in filaments in liquid cultures (Fig. 1). Longer filaments were observed in solid cultures.
The sequence of the 16S rRNA gene determined for strain FM1025T was compared to 16S rRNA from ABI. For identification of closest relatives, the Biosystems) using the 16S rRNA full gene sequencing kit sequenced on an ABI 310 sequencer (Applied Qiagen PCR purification kit and both strands were PCR using primers Fpna1 (5'-GTTTA-3') and RPna2 (5'-CAAACCCGAAGCCGGCTG-3'). Amplified DNA fragments were purified using the Qiagen PCR purification kit and both strands were sequenced on an ABI 310 sequencer (Applied Biosystems) using the 16S rRNA full gene sequencing kit from ABI. For identification of closest relatives, the sequence of strain FM1025T was compared to 16S rRNA gene sequences in GenBank using BLASTN version 2.2.17 (Altschul et al., 1997). Sequence alignment was performed using CLUSTAL W. Phylogenetic trees were constructed using PAUP 4.0, on 1399 base comparisons using the neighbour-joining method and bootstrap values of 1000 replicates. The sequence of the 16S rRNA gene determined for strain FM1025T (1399 nt) placed it near the genus Megamonas within the phylum Firmicutes, in the lineage Clostridia, Clostridiales, Acidaminococcaceae (Morotomi et al., 2007). The closest relatives were Megamonas hypermegale DSM 1672T, at 94.9 % similarity and 71 nucleotide differences, and the recently described Megamonas funiformis YIT 11815T (Sakon et al., 2008), at 97.6 % similarity and 29 nucleotide differences. The 100 % bootstrap value observed strongly supports the grouping of FM1025T with Megamonas hypermegale, the type species of the genus (Cato & Barnes, 1976; Harrisson & Hansen, 1963; Shah & Collins, 1982). Sequence similarity of less than 91 % was observed with representative type strains of Pectinatus and Selenomonas species (Fig. 2).

This difference in 16S rRNA gene sequence suggests a phyllogenetic difference sufficient to assign strain FM1025T to a novel species in the genus Megamonas. This is supported by the DNA G+C content of 33 ± 2 mol% (Shah & Collins, 1982). However strain FM1025T (1.0–6.0 μm) can be easily differentiated from M. hypermegale (2–4.6× 11.6–15 μm) by its smaller cells (Cato & Barnes, 1976; Harrisson & Hansen, 1963) and on the basis of an absence of acid production from melezitose and l-rhamnose. It can be differentiated from M. funiformis (Sakon et al., 2008) on the basis of the presence of acid production from glycerol and cellobiose. 16S rRNA gene sequence comparisons revealed similarity above 98 % with numerous sequences of uncultured isolates from the caeca of broiler chickens (Bjerrum et al., 2006) or other types of organisms. Strain FM1025T is the first isolate of this taxon to be cultivated and described.

API 20A profiles were analysed after 24 and 48 h and submitted to bioMérieux for identification. The profiles obtained were similar to those of the Gram-negative Prevotella ruminicola subsp. ruminicola and the Gram-positive Actinomyces israelii (80.2 % ID in both cases). Sensitivity towards antibiotics was tested using disc assays. A 24 h culture in TGY broth was diluted to an OD600 of 0.12 as specified by the recommendations of the Comité de l’Antibiogramme of the Société Française de Microbiologie. TGY agar plates were flooded with 2 ml of this dilution prior to placing the antibiotic discs (Bio-Rad). Inhibition haloes were measured after 24 h incubation in the anaerobic chamber. Strain FM1025T was resistant to vancomycin at 30 μg, similar to some Prevotella, Bacteroides and Fusobacterium species and Clostridium innocuum. Strain FM1025T was sensitive to 25 μg amoxicillin, 10 μg imipenem, 4 μg metronidazole, 30 μg chloramphenicol, 20 μg amoxicillin and 10 μg clavulanic acid.

Static TGY broth cultures (18 ml) were used for fermentation analysis. Anaerobic jars and long glass tubes were preferred to the anaerobic chamber and Erlenmeyer flasks in order to minimize the loss of volatile by-products. Aliquots of cultures (48 h, 37 °C) were centrifuged for 5 min at 7500 g. Culture supernatants were sterile-filtered (0.22 μm) and analysed in triplicate using a gas chromatograph (Hewlett Packard 5890A) as described by Erable et al. (2005). Glucose concentration was evaluated by the DNS.

Fig. 1. Scanning electron micrographs of cells of strain FM1025T grown anaerobically for 48 h in TGY broth. The arrow indicates a filamentous structure. Bars, 20 μm (top) and 2 μm (bottom).
We were unable to detect the presence of lactate, propanol, acetone, butyrate, 2,3-butanediol or pentanoate in the supernatant of strain FM1025T. Hence, FM1025T can also be differentiated from *M. funiformis* (Sakon et al., 2008) and *M. hypermegale* (Harrisson & Hansen, 1963) by the absence of lactate as an end product from TYG broth. Acetate and propionate were the only organic acids detected from glucose fermentation. Propionate appeared to be the major fermentation end product, with a concentration of 25 mM after 48 h compared with 20 mM for acetate. The initial concentration of glucose was 28 mM and reached 1.3 mM after 72 h of culture. Considering that 90% of the glucose consumed (26.3 mM) was converted into end products (the rest being converted into biomass), we assessed that 95% was fermented into acetate and propionate. This confirmed that acetate and propionate were the major organic acids produced by strain FM1025 T in TGY medium. Moreover, the final pH of cultures was 4.6–4.9, corresponding to the pKₐ of acetate (pKₐ = 4.75) and propionate (pKₐ = 4.87).

**Description of Megamonas rupellensis sp. nov.**

*Megamonas rupellensis* (ru.pel.len’sis. M.L. fem. adj. *rupellensis* referring to La Rochelle, the site of isolation of the type strain).

Obligately anaerobic, mesophilic, saccharophilic, non-spore-forming, non-motile, Gram-negative or Gram-variable, long rods (1.0–6.0 μm). Cells occur singly, in pairs or in chains in liquid medium. Growth occurs in TGY broth within 24 h. On solid medium, colonies are ‘ecru’ and notched, 10–20 mm in diameter after 2 days of growth at 37 °C. End products are acetate and propionate. The pH for growth is pH 5.0–9.0, with an optimum at pH 7.0. Oxidase and catalase activities are absent. Nitrate and nitrite reduction activities are absent. Acids are produced from D-glucose, D-mannitol, D-lactose, sucrose, maltose, salicin, D-xylene, L-arabinose, glycerol, cellobiose, D-mannose, raffinose, D-sorbitol and trehalose, but not from melezitose or L-rhamnose. Resistant to vancomycin (30 μg per disc), but not to amoxicillin (25 μg), imipenem (10 μg), metronidazole (4 μg), chloramphenicol (30 μg), amoxicillin (20 μg) or clavulanic acid (10 μg). The G+C content of DNA of the type strain is 33 ± 2 mol%.

The type strain, FM1025 T (=DSM 19944 T =CIP 109788 T), was isolated from the caecum of a duck in La Rochelle, France.

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**References**


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**Fig. 2.** Phylogenetic tree showing the position of strain FM1025 T, other species of the ‘*Acidaminococcaceae*’ and *Megamonas hypermegale* DSM 1672 T, based on 16S rRNA gene sequence similarities. The tree was constructed by using the neighbour-joining method, with bootstrap values (reliability values of internal branches) expressed as percentages of 1000 replicates. Accession numbers are shown in parentheses. Bar, 1% sequence divergence. *Anaerobacter polyendosporus* DSM 5272 T was used as the outgroup.


