***Coenonia anatina* gen. nov., sp. nov., a novel bacterium associated with respiratory disease in ducks and geese**

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Taxon 1502 was originally described as a *Riemerella anatipestifer*-like bacterium causing exudative septicaemia in ducks and geese. In the present study, an integrated genotypic and phenotypic approach was used to elucidate the phylogenetic affiliation and taxonomic relationships of 12 strains of taxon 1502. Whole-cell protein and fatty acid analyses and an extensive biochemical examination by using conventional tests and several API microtest systems indicated that all isolates formed a homogeneous taxon, which was confirmed by DNA–DNA hybridizations. 16S rDNA sequence analysis of a representative strain (LMG 14382^T) indicated that this taxon belongs to the *Cytophaga–Flavobacterium–Bacteroides* phylum and revealed a moderate but distinct relationship to species of the genus *Capnocytophaga* (overall 16S rDNA sequence identities were 88.8–90.2%). Taxon 1502 is concluded to represent a single species that should be allocated to a novel genus, and the name *Coenonia anatina* gen. nov., sp. nov. is proposed. The DNA G+C content of representative strains was 35–36 mol% and the type strain is LMG 14382^T.

Keywords: *Coenonia anatina* gen. nov., sp. nov., *Capnocytophaga*, taxon 1502, poultry

INTRODUCTION

Riemerella anatipestifer is a globally distributed agent of septicaemic disease in domestic and wild ducks and is of major economic importance (Brogden, 1989). In a recent study, Hinz *et al.* (1998) described various phenotypic characteristics of about 200 *R. anatipestifer* and *R. anatipestifer*-like bacteria isolated mainly in Germany from various species of birds and swine. Sixty-four of these isolates, 59 of which were obtained from Pekin ducks, two from muscovy ducks and three from geese, differed considerably from all other strains examined (Hinz *et al.*, 1998) and were referred to as *R. anatipestifer*-like bacteria of the taxon 1502 [1502 being the number of a representative strain (= LMG 14382^T)].

In the present study, 12 randomly selected strains of taxon 1502 were used to elucidate the phylogenetic

affiliation and taxonomic relationships of this taxon by means of an integrated genotypic and phenotypic approach. In the course of the study, members of the genus *Capnocytophaga* were shown to be the closest relatives of taxon 1502 strains. Therefore, representative strains of the various *Capnocytophaga* species characterized in a previous polyphasic taxonomic study (Vandamme *et al.*, 1996) and of *R. anatipestifer* were included as references throughout the study.

METHODS

Bacterial strains and growth conditions. Unless specified otherwise, strains were grown on Trypticase Soy agar (BBL) and incubated at 36–37 °C in a microaerobic atmosphere containing approximately 5% O₂, 3.5% CO₂, 7.5% H₂ and 84% N₂. The strains and their sources are listed in Table 1. Bacteriological purity was checked by plating and examining living and Gram-stained cells.

Whole-cell protein analysis. Strains were grown for 2 d. Preparation of cellular protein extracts, PAGE, densitometric analysis, normalization and interpolation of the protein profiles and numerical analysis were performed as

The EMBL accession number for the 16S rDNA sequence of strain LMG 14382^T reported in this paper is Y17612.

Table 1. List of strains studied

Strain	Other strain no.*	Received from	Source
<i>Coenonia anatina</i> LMG 14382 ^T	1502-91 ^T	(Own isolate)	Pekin duck (Germany, 1991)
<i>Coenonia anatina</i> LMG 14383	727-82	(Own isolate)	Pekin duck (Germany, 1982)
<i>Coenonia anatina</i> LMG 14384	726-82	(Own isolate)	Muscovy duck (Germany, 1982)
<i>Coenonia anatina</i> LMG 17807	481-85	(Own isolate)	Goose (Germany, 1985)
<i>Coenonia anatina</i> LMG 17808	599-78	(Own isolate)	Muscovy duck (Germany, 1978)
<i>Coenonia anatina</i> LMG 17809	461-84	(Own isolate)	Goose (Germany, 1984)
<i>Coenonia anatina</i> LMG 17810	G1219-92	(Own isolate)	Pekin duck (Germany, 1992)
<i>Coenonia anatina</i> LMG 17811	G700-94	(Own isolate)	Pekin duck (Germany, 1994)
<i>Coenonia anatina</i> LMG 17812	G1382-93	(Own isolate)	Pekin duck (Germany, 1993)
<i>Coenonia anatina</i> LMG 17813	G31-94	(Own isolate)	Pekin duck (Germany, 1994)
<i>Coenonia anatina</i> LMG 17814	G356-92	(Own isolate)	Pekin duck (Germany, 1992)
<i>Coenonia anatina</i> LMG 17815	G157-93	(Own isolate)	Pekin duck (Germany, 1993)
<i>Capnocytophaga canimorsus</i> LMG 11510	CCUG 12569	CCUG	Human, blood (USA, 1965)
<i>Capnocytophaga cynodegmi</i> LMG 11513 ^T	CCUG 24742 ^T	CCUG	Dog, mouth (USA, 1979)
<i>Capnocytophaga gingivalis</i> LMG 11514 ^T	CCUG 9715 ^T	CCUG	Periodontitis (USA, 1978)
<i>Capnocytophaga granulosa</i> LMG 16022 ^T	JCM 8566 ^T	JCM	Supragingival dental plaque (Japan)
<i>Capnocytophaga haemolytica</i> LMG 16021 ^T	JCM 8565 ^T	JCM	Supragingival dental plaque (Japan)
<i>Capnocytophaga ochracea</i> LMG 11516	CCUG 9972	CCUG	Bülow drain secretion (Sweden, 1980)
<i>Capnocytophaga sputigena</i> LMG 11518 ^T	CCUG 9714 ^T	CCUG	Periodontitis (USA, 1978)
<i>Ornithobacterium rhinotracheale</i> LMG 9086 ^T	CCUG 23171 ^T	CCUG	Turkey, respiratory tract (UK)
<i>Riemerella anatipestifer</i> LMG 11054 ^T	CCUG 21370 ^T	CCUG	Duck, blood (USA)

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described by Pot *et al.* (1994) using the GELCOMP 4.0 software package (Applied Maths). The profiles were recorded and stored on a PC. The similarity between all pairs of traces was expressed by the Pearson product moment correlation coefficient converted, for convenience, to a percentage value.

Fatty acid methyl ester analysis. Strains were grown for 48 h and then a loopful of well-grown cells was harvested. Preparation, separation, identification and numerical comparison of the fatty acid methyl esters was performed using the Microbial Identification System (Microbial ID) as described before (Vandamme *et al.*, 1992).

Preparation of high-molecular-mass DNA. High-molecular-mass native DNA was prepared as described before (Vandamme *et al.*, 1992).

DNA base compositions. All DNA G+C contents were determined by thermal denaturation and calculated by using the equation of Marmur & Doty (1962), as modified by De Ley (1970).

DNA-DNA hybridization experiments. The degree of DNA-DNA binding, expressed as a percentage, was determined spectrophotometrically by the initial renaturation rate method of De Ley *et al.* (1970). Each value is the mean of at least two hybridization experiments. Values of 30% DNA binding or less do not represent significant DNA homology. The total DNA concentration was about 52 µg ml⁻¹ and the optimal renaturation temperature in 1 × SSC (0.15 M NaCl, 0.015 M sodium citrate, pH 7) was 60.3 °C.

16S rDNA sequencing. *In vitro* amplification and direct

sequencing of 16S rRNA-encoding DNA fragments was done as described previously (Springer *et al.*, 1992). The new 16S rRNA sequence was fitted into an alignment of about 12000 homologous, full and partial primary structures available in public databases (Ludwig, 1995) using the respective automated tools of the ARB software package (Ludwig & Strunk, 1996). Distance matrix, maximum-parsimony and maximum-likelihood methods were applied, as implemented in the ARB software package. Different data sets, varying with respect to the outgroup reference organisms included (sequences) as well as alignment positions, were analysed (Ludwig *et al.*, 1998).

Phenotypic tests. Classical biochemical tests were performed as described before (Vandamme *et al.*, 1998). The basal medium used was Columbia agar (Oxoid) supplemented with 7% defibrinated sheep blood; microaerobic and anaerobic growth conditions were generated by means of the Oxoid *Campylobacter* gas-generation kit and Anaerocult-A system (Merck), respectively. The following characters were examined: growth on MacConkey agar, Simmons citrate agar and litmus lactose agar; presence of phenylalanine deaminase, oxidase, catalase, urease, ornithine and lysine decarboxylase, arginine dihydrolase, hyaluronidase and chondroitin sulfatase activity; indole production; hydrolysis of gelatin and aesculin; nitrate reduction; utilization of malonate as carbon source; and methyl red and Voges-Proskauer reaction (production of acetylmethylcarbinol).

Acid production from carbohydrates was tested by using the conventional test system and buffered single-substrate procedure as described before (Vandamme *et al.*, 1998). The API 20NE, API ZYM and API ID 32 E microtest systems

were used according to the recommendations of the manufacturer (bioMérieux).

RESULTS

PAGE of whole-cell proteins

Duplicate protein extracts of several strains were prepared to check the reproducibility of the growth conditions and the preparation of the extracts. The correlation between duplicate protein patterns was >94%. All 12 taxon 1502 strains examined had virtually identical whole-cell protein patterns, which clustered above a correlation of 91% (Fig. 1).

Fatty acid methyl ester composition

The mean whole-cell fatty acid composition of the 12 strains of taxon 1502 is given in Table 2. It typically comprised 13:0 iso (about 34%), 14:0 (about 3%), 15:0 iso (about 41%), 15:0 anteiso (about 3%), 15:0 iso 3-OH (about 6.5%), 16:0 3-OH (about 4%) and 17:0 iso 3-OH (about 6.5%).

DNA base composition

The DNA G+C content of strains LMG 14382^T, LMG 17808 and LMG 17809 was 35, 35 and 35.5 mol%, respectively.

Fig. 1. Dendrogram derived from the unweighted pair group mean linkage of correlation coefficients between whole-organism protein patterns of all strains examined.

Table 2. Fatty acid composition of the strains studied

The number of strains of each taxon examined is indicated. Those fatty acids for which the mean amount for all taxa was less than 1% are not shown. Therefore, the percentages for each group do not total 100. Mean percentages $\pm$ standard deviations are given. TR, Trace amount (<1%); ND, not detected. ECL, equivalent chain-length value. Data for *Capnocytophaga* species were from Vandamme *et al.* (1996).

Taxon (n)	13:0 iso	ECL 13:566	14:0	15:0 iso	15:0 anteiso	15:0	16:0	15:0 iso 3-OH	ECL 16:580	16:0 3-OH	17:0 iso 3-OH
<i>Coenonia anatina</i> (12)	33.9 $\pm$ 4.6	TR	2.8 $\pm$ 2.4	40.8 $\pm$ 4.7	3.2 $\pm$ 0.7	ND	TR	6.4 $\pm$ 1.3	ND	4.1 $\pm$ 0.9	6.3 $\pm$ 2.3
<i>Riemerella anatipestifer</i> (16)*	14.9 $\pm$ 3.6	1.5 $\pm$ 0.6	ND	52.8 $\pm$ 4.9	5.8 $\pm$ 1.8	ND	TR	8.0 $\pm$ 2.8	TR	ND	13.1 $\pm$ 3.5
<i>Ornithobacterium rhinotracheale</i> (21)†	TR	4.2 $\pm$ 1.5	TR	57.4 $\pm$ 6.1	TR	ND	2.9 $\pm$ 1.3	8.1 $\pm$ 1.9	1.1 $\pm$ 0.5	2.8 $\pm$ 1.8	20.2 $\pm$ 5.0
<i>Capnocytophaga canimorsus</i> (5)	1.3 $\pm$ 0.5	2.2 $\pm$ 0.8	1.7 $\pm$ 0.1	66.7 $\pm$ 1.7	1.4 $\pm$ 0.5	ND	2.3 $\pm$ 0.5	6.6 $\pm$ 1.6	TR	4.3 $\pm$ 0.7	11.5 $\pm$ 0.4
<i>Capnocytophaga cynodegmi</i> (4)	1.0 $\pm$ 0.4	1.9 $\pm$ 0.7	2.2 $\pm$ 0.7	66.5 $\pm$ 4.7	1.3 $\pm$ 0.5	ND	2.8 $\pm$ 0.4	3.9 $\pm$ 0.6	1.0 $\pm$ 0.2	4.6 $\pm$ 2.5	12.9 $\pm$ 3.6
<i>Capnocytophaga gingivalis</i> (3)	3.9 $\pm$ 0.7	1.7 $\pm$ 0.3	1.0 $\pm$ 0.1	63.5 $\pm$ 4.9	1.8 $\pm$ 0.2	1.6 $\pm$ 0.9	1.9 $\pm$ 1.1	2.6 $\pm$ 1.1	1.2 $\pm$ 0.3	5.7 $\pm$ 1.5	14.0 $\pm$ 2.2
<i>Capnocytophaga ochracea</i> (6)	2.1 $\pm$ 0.5	1.3 $\pm$ 0.4	1.5 $\pm$ 0.8	63.5 $\pm$ 9.3	1.6 $\pm$ 1.0	TR	3.6 $\pm$ 2.3	1.2 $\pm$ 0.4	TR	8.1 $\pm$ 6.6	13.8 $\pm$ 2.8
<i>Capnocytophaga sputigena</i> (5)	2.9 $\pm$ 1.0	1.2 $\pm$ 0.9	2.2 $\pm$ 1.0	68.0 $\pm$ 2.1	1.9 $\pm$ 0.3	TR	4.2 $\pm$ 1.5	1.2 $\pm$ 0.4	TR	5.9 $\pm$ 1.2	9.7 $\pm$ 2.9
<i>Capnocytophaga granulosa</i> (5)	2.1 $\pm$ 1.0	TR	1.9 $\pm$ 1.2	75.5 $\pm$ 6.8	1.3 $\pm$ 0.3	TR	4.2 $\pm$ 4.0	1.6 $\pm$ 0.5	TR	5.0 $\pm$ 2.7	6.5 $\pm$ 1.7
<i>Capnocytophaga haemolytica</i> (1)	TR	TR	2.2	60.7	1.8	TR	9.8	TR	TR	8.4	9.4

* Data from Segers *et al.* (1993).

† Data from Vandamme *et al.* (1994). *O. rhinotracheale* strains also contain 1.5 $\pm$ 1.0% 17:0 iso.

Fig. 2. 16S rRNA-based tree reflecting the phylogenetic relationships of strain LMG 14382^T and a selection of related species and groups. A maximum-likelihood tree was reconstructed from a data set of 160 almost-complete 16S rRNA sequences from representatives of the *Cytophaga-Flavobacterium-Bacteroides* phylum. Only alignment positions which share identical nucleotides in at least 50% of the entire data-set of all available 16S rRNA sequences from the phylum were included for calculations. The tree topology was corrected according the results of distance-matrix and maximum-parsimony analyses of all available 16S rRNA sequences from the phylum or the full data-set of 12 000 sequence entries, respectively. Multifurcations indicate that a relative branching order could not be determined unambiguously or a common branching order was not supported when applying different treeing methods on varying data sets. The bar indicates 10% estimated sequence divergence. The following strains were used as references (accession numbers are given in parentheses): *Psychroserpens burtonensis* ACAM181 (U62912), *Gelidibacter algens* A296 (U62916), *Cytophaga marinoflava* ATCC 19326^T (M58770), *Flavobacterium gondwanense* ATCC 51278^T (M92278), *Flavobacterium salegens* DSM 5424^T (M92279), *Cytophaga latercula* ATCC 23177^T (M58769), *Polaribacter filamentus* 215 (U73726), *Flexibacter maritimus* (strain is not specified) (M58780), *Cytophaga uliginosa* ATCC 14397^T (M28238) and NCIMB 1863^T (M62799), *Cytophaga lytica* ATCC 23178^T (M28058 and M62796), *Capnocytophaga ochracea* ATCC 33596^T (X67610), *Capnocytophaga sputigena* ATCC 33612^T (X67609), *Capnocytophaga haemolytica* LMG 16021^T (U41349), *Capnocytophaga canimorsus* ATCC 35979^T (L14637), *Capnocytophaga cynodegmi* ATCC 49044^T (L14638), *Capnocytophaga granulosa* LMG 12119 (U41348), *Capnocytophaga gingivalis* LMG 12118 (U41346), *Flavobacterium columnare* ATCC 43622 (M58781), *Flavobacterium johnsoniae* ATCC 17061^T (M59051), *Flavobacterium flevense* ATCC 27944^T (M58767), *Flavobacterium aquatile* ATCC 11947^T (M28236 and M62797), *Myroides odoratus* NCTC 11036^T (M58777), *Chryseobacterium indologenes* ATCC 29897^T (M58773), *Chryseobacterium gleum* ATCC 35910^T (M58772), *Bergeyella zoohelcum* ATCC 43767^T (M93153), *Chryseobacterium balustinum* ATCC 33487^T (M58771), *Chryseobacterium indoltheticum* ATCC 27950^T (M58774), *Riemerella anatipestifer* ATCC 11845^T (U10877), *Empedobacter brevis* ATCC 14234 (M59052), *Weeksella virosa* ATCC 43766^T (M93152) and *Ornithobacterium rhinotracheale* LMG 9086^T (L19156).

DNA-DNA hybridization results

The DNA-DNA binding level between LMG 14382^T and LMG 17808 was 55% and the DNA-DNA binding level between LMG 14382^T and LMG 17809 was 85%.

Phylogenetic analysis of the 16S rDNA sequence

16S rRNA-encoding DNA from strain LMG 14382^T was amplified *in vitro* and sequenced directly. A comparative database analysis revealed a moderate but distinct relationship to the species of the genus *Capnocytophaga* (Fig. 2). The overall 16S rRNA sequence identities were 88.8–90.2%. These species represent a monophyletic sub-group of the bacterial phylum *Cytophaga-Flavobacterium-Bacteroides*, containing strain LMG 14382^T as its deepest branch.

Neighbour groups comprise species of the genera *Ornithobacterium*, *Riemerella*, *Gelidibacter*, *Myroides*, *Polaribacter* and *Psychroserpens* as well as members of the phylogenetically heterogeneous genus *Flexibacter* and the emended genera *Flavobacterium* and *Cytophaga* and various taxa previously classified as flavobacteria or cytophagas (Vandamme *et al.*, 1994; Bernardet *et al.*, 1996; Vancanneyt *et al.*, 1996; Nakagawa & Yamasoto, 1996). The corresponding overall sequence identities for strain LMG 14382^T and the representatives of these groups were 88.6% and lower.

Phenotypic tests

Phenotypic tests were performed on all taxon 1502 strains and the reference *Capnocytophaga* strains included in Table 1. When tests were performed in

Table 3. Differential phenotypic characteristics of all strains examined

Symbols used: +, characteristic present; –, characteristic absent; (+), characteristic weakly present or reaction delayed; v, strain-dependent reaction. In the case of strain-dependent reactions, the number of strains giving a positive reactions is given in parentheses. *Capn.*, *Capnocytophaga*.

Characteristic	<i>Coenonia anatina</i> (12 strains)	<i>Capn. canimorsus</i> LMG 11510	<i>Capn. cynodegmi</i> LMG 11513 ^T	<i>Capn. gingivalis</i> LMG 11514 ^T	<i>Capn. granulosa</i> LMG 16022 ^T	<i>Capn. haemolytica</i> LMG 16021 ^T	<i>Capn. ochracea</i> LMG 11516	<i>Capn. sputigena</i> LMG 11518 ^T
Aerobic growth	–	–	(+)	–	(+)	–	–	–
Anaerobic growth	(+)	–	–	(+)	(+)	(+)	+	(+)
Growth on litmus lactose agar in microaerobic conditions	–	–	–	+	(+)	(+)	–	(+)
Catalase activity	+	+	+	–	–	–	–	–
Oxidase activity	+	+	+	+	+	–	–	–
Urease activity	–	+	–	–	–	–	–	–
Nitrate reduction (classical method)	–	–	–	–	–	+	–	–
Nitrate reduction (API 20 NE):								
Formation of nitrite	–	–	–	–	–	+	–	–
Denitrification	–	–	–	–	–	+	–	–
Voges-Proskauer reaction	v (11)	–	–	–	–	–	–	–
Hydrolysis of aesculin:								
API 20 NE	v (10)	+	+	(+)	+	+	+	+
Conventional test	v (5)	–	+	+	+	+	+	+
Gelatinase activity	–	+	–	–	–	–	(+)	(+)
Hyaluronidase activity	+	–	–	–	–	–	–	–
Chondroitin sulfatase activity	+	–	–	–	–	–	–	–
Acid production (using BSS test) from:								
D-Fructose	v (10)	–	+	–	+	–	+	+
Lactose	+	+	+	–	+	+	+	+
D-Galactose	+	–	+	–	+	–	+	+
N-Acetyl D-glucosamine	+	+	+	–	–	+	+	+
Lactulose	+	+	+	–	+	+	+	+
Saccharose	–	+	+	+	+	+	+	+
Salicin	–	–	(+)	(+)	–	–	+	+
Sorbitose	–	–	+	+	–	+	(+)	+
Glucose fermentation (API 20 NE)	v (6)	(+)	+	–	–	(+)	+	–
β -Galactosidase activity (API 20 NE)	+	+	+	–	+	+	+	+
Activity of (using API ZYM):								
Esterase C4	+	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Ester lipase C8	+	(+)	+	+	(+)	+	+	(+)
Leucine arylamidase	+	+	+	+	+	–	+	+
Valine arylamidase	v (10)	+	+	+	+	–	+	+
Cystine arylamidase	v (6)	+	+	+	+	–	+	+
Trypsin	+	+	+	+	–	+	–	–
Chymotrypsin	–	–	–	–	–	–	(+)	–
Naphthol-AS-B1-phosphohydrolase	+	–	+	+	+	+	+	+
α -Galactosidase	v (1)	–	+	–	–	–	–	–
β -Galactosidase	v (8)	–	–	–	+	–	+	+
α -Glucosidase	v (11)	+	+	+	+	+	+	+
β -Glucosidase	–	–	–	–	+	–	+	–
N-Acetyl- β -glucosaminidase	+	+	+	–	–	+	+	+
α -Fucosidase	v (11)	(+)	+	–	–	–	–	–
Activity of (using API ID 32 E):								
Lipase	+	–	(+)	–	(+)	+	+	–
β -Galactosidase	+	+	+	–	+	(+)	+	+
N-Acetyl- β -glucosaminidase	+	+	+	–	–	+	+	+
Acidification of (using API ID 32 E):								
Phenol red	+	–	–	–	–	(+)	+	+
Maltose	–	–	–	–	–	–	+	+
Adonitol	–	–	–	–	–	–	+	–
Palatinose	–	–	–	–	–	+	+	+
Glucose	v (8)	–	–	–	–	+	+	+
Saccharose	–	–	–	–	–	+	+	+
Cellobiose	–	–	+	–	–	+	+	+

multiple API microtest systems or in a conventional way and by means of API microtest systems, identical results were usually obtained. Discrepancies were however observed for aesculin hydrolysis [present in 17 strains when examined by using API 20NE; absent in eight strains when performed by using the classical method (aesculin broth; Merck)], gelatinase activity [absent in all strains when examined by using API 20NE; present or weakly present in three strains when

examined by using the classical method (gelatin agar: standard I nutrient agar, Merck catalogue no. 7881, supplemented with 0.4 % gelatin)], acidification of maltose, glucose (always present when using the BSS test; variable reactions when using the API ID 32 E microtest system) and saccharose (variable reactions when using the BSS test, but less-positive results when using the API ID 32 E microtest system) and activity of α -glucosidase, β -glucosidase, α -galactosidase (always

present when using the API ID 32 E microtest system, variable reactions when using the API ZYM microtest system), *N*-acetyl- β -glucosaminidase and β -galactosidase (variable reactions when using the API ID 32 E microtest system, but less-positive results when using the API ZYM microtest system). Clearly, the API ZYM gallery seems to have a lower detection sensitivity than the other microtest systems used.

The following characters were present in all of the strains examined: growth on blood agar in microaerobic conditions; acid production from D-glucose, maltose, D-mannose and dextrin in BSS medium; activity of alkaline and acid phosphatase as determined by using the API ZYM microtest system; aesculin hydrolysis as determined by using the API 20NE microtest system (one strain, *Capnocytophaga gingivalis* LMG 11514^T, gave a weak reaction); and activity of α -glucosidase, β -glucosidase (two strains of taxon 1502 gave negative reactions; one strain, *Capnocytophaga gingivalis* LMG 11514^T, gave a weak reaction), α -galactosidase, α -maltosidase and L-aspartic acid arylamidase as determined by using the API ID 32 E microtest system.

The following characters were absent in all of the strains examined: growth on MacConkey agar in microaerobic conditions; utilization of citrate; indole production; growth on Simmons citrate agar; methyl red reaction; activity of arginine dihydrolase, ornithine and lysine decarboxylase; alkali from malonate and phenylalanine deaminase activity (as determined by using classical phenotypic tests); acid production from trehalose, adonitol, L-arabinose, dulcitol, *myo*-inositol, D-mannitol, D-sorbitol and D-xylose in BSS medium; indole production, activity of arginine dihydrolase and urease, liquefaction of gelatin and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, maltose, D-glucuronate, caprate, adipate, L-malate, citrate and phenyl acetate as determined by using the API 20NE microtest system; activity of lipase C14, β -glucuronidase and α -mannosidase as determined by using the API ZYM microtest system; and activity of arginine dihydrolase, ornithine and lysine decarboxylase, urease and β -glucuronidase, indole production and acidification of L-arabitol, galacturonate, 5-ketogluconate, mannitol, L-arabinose, D-arabitol, trehalose, rhamnose, inositol and sorbitol as determined by using the ID 32 E microtest system.

All other tests results were variable amongst strains or amongst taxa and are presented in Table 3.

DISCUSSION

Phylogenetic allocation of taxon 1502 bacteria

In the present study, a collection of 12 strains of taxon 1502 bacteria (Hinz *et al.*, 1998) was examined by a polyphasic taxonomic approach. Although initially identified as *R. anatipestifer*-like bacteria, these strains were reported to differ in not less than 17 biochemical

tests from typical *R. anatipestifer* strains, which indicated strongly that they represented a distinct taxon. Whole-cell protein (Fig. 1) and fatty acid analysis (Table 2) of 12 strains of taxon 1502 indicated that these isolates formed a single species, which was confirmed by DNA–DNA binding studies between strain LMG 14382^T and two additional isolates, selected at random (values of 55 and 85% were measured).

Comparative 16S rDNA sequence analyses applying alternative treeing methods upon various data sets clearly supported the phylogenetic position of strain LMG 14382^T as a member of the *Cytophaga*–*Flavobacterium*–*Bacteroides* phylum of the *Bacteria*. This strain is affiliated to the species of the genus *Capnocytophaga* but significantly separated from the latter and other microaerophilic and capnophilic organisms such as *Ornithobacterium* and *Riemerella* species (Fig. 2). Whereas no stable relative branching order could be found for the phylogenetic sub-groups of *Capnocytophaga*, the separate status of strain LMG 14382^T was evident in all treeing analyses. The tree topologies, in combination with the rather low overall 16S rRNA sequence identities of strain LMG 14382^T and the other representatives of the phylum, support the description of the isolate as the representative of a new genus and species. The name *Coenonia anatina* gen. nov., sp. nov. is proposed below.

Identification of *Coenonia anatina*

Phenotypically, *Coenonia anatina* (taxon 1502) was reported to be more similar to *Ornithobacterium rhinotracheale* [another member of the *Cytophaga*–*Flavobacterium*–*Bacteroides* phylum that is frequently isolated from poultry specimens (Vandamme *et al.*, 1994)] than to *R. anatipestifer* (Hinz *et al.*, 1998). Hinz *et al.* (1998) described a variety of differential biochemical tests to distinguish between these three taxa. The most important characters that allow the differentiation of *Coenonia anatina* from *R. anatipestifer* are the absence of arginine dihydrolase and gelatinase activity, the presence of hyaluronidase and chondroitin sulfatase activity, aesculin hydrolysis and the presence of β -glucosaminidase activity (as determined by using the API ZYM microtest system). *Coenonia anatina* strains are readily differentiated from *O. rhinotracheale* strains by the presence of catalase activity and the absence of urease and arginine dihydrolase activity. Features that allow the differentiation of *Coenonia anatina* from *Capnocytophaga* species (its closest phylogenetic neighbours) are a positive Voges–Proskauer reaction, the presence of hyaluronidase and chondroitin sulfatase activity and absence of α -glucosidase activity (as determined using the API ZYM microtest system). A variety of additional tests differentiating *Coenonia anatina* from individual *Capnocytophaga* species is listed in Table 3.

In addition, whole-cell protein (Fig. 1) and fatty acid analysis (Table 2) allow ready differentiation of

Coenonia anatina from species belonging to the genera *Riemerella*, *Ornithobacterium* and *Capnocytophaga*. The cellular fatty acid of *Coenonia anatina* strains is primarily composed of a variety of branched-chain fatty acids, which is typical for members of this phylogenetic lineage.

Description of *Coenonia* gen. nov.

Coenonia (coe.no'ni.a. Gr. n. *coenonia* community, association; N.L. fem. n. *Coenonia* refers to the association between these bacteria and a host).

Coenonia cells are Gram-negative, non-spore-forming, microaerophilic, non-motile, rod-shaped bacteria. The major fatty acid components of all strains examined are branched-chain fatty acids, including 13:0 iso, 15:0 iso, 15:0 anteiso, 15:0 iso 3-OH, 16:0 3-OH and 17:0 iso 3-OH. The DNA G+C content of representative strains was 35–36 mol% and the type species is *Coenonia anatina* sp. nov.

Description of *Coenonia anatina* sp. nov.

Coenonia anatina (a.na.ti'na. L. adj. *anatinus* of a duck).

Cells are Gram-negative, non-spore-forming rods. Non-motile. In 16-h-old cultures on blood agar, the mean cell size is 0.2–0.4 µm in width and 1.25–2.5 µm in length. Some cells are spherically swollen, spindle- or lemon-shaped and arranged in short chains. Strains produce flat-convex, circular and non-pigmented to whitish colonies with entire edges and a smooth surface on blood agar. The strains do not require special growth factors and grow on conventional media. Growth is optimal at 37 °C in a microaerobic and CO₂-enriched atmosphere. Growth is weak in anaerobic conditions and absent in aerobic conditions. No growth on MacConkey agar or on Simmons citrate agar. Non-haemolytic. All strains produce catalase and oxidase activity. Hyaluronidase and chondroitin sulfatase activity is present. No urease, gelatinase, arginine dihydrolase, phenylalanine deaminase or ornithine or lysine decarboxylase activity. No reduction of nitrate. Hydrolysis of aesculin. Production of acetylmethylcarbinol but not indole. Methyl red reaction is negative. No utilization of citrate. No alkali from malonate. Acid production in BSS medium from D-glucose, D-fructose, maltose, D-mannose, lactose, D-galactose, *N*-acetyl glucosamine, lactulose and dextrin but not from trehalose, adonitol, L-arabinose, dulcitol, *myo*-inositol, D-mannitol, D-sorbitol, saccharose, salicin, sorbose or D-xylose. Using the API ZYM microtest system, activities of alkaline and acid phosphatase, esterase C4, ester lipase C8, leucine, valine and cystine arylamidase, naphthol-AS-B1-phosphohydrolase, β-galactosidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, α-fucosidase and trypsin are detected; activity of lipase C14, chymotrypsin, β-glucuronidase, α-galactosidase, β-glucosidase and α-mannosidase are not detected. Using the API ID 32 E microtest system,

acidification of glucose and activities of α-glucosidase, β-glucosidase, α-galactosidase, β-galactosidase, α-maltosidase, lipase, *N*-acetyl-β-glucosaminidase and L-aspartic acid arylamidase are detected; activities of arginine dihydrolase, ornithine and lysine decarboxylase, urease and β-glucuronidase, indole production and acidification of L-arabitol, galacturonate, 5-keto-glucuronate, mannitol, L-arabinose, D-arabitol, trehalose, rhamnose, inositol, maltose, adonitol, palatinose, saccharose, cellobiose and sorbitol are not detected. The major fatty acid components of all strains examined are 13:0 iso (about 34%), 14:0 (about 3%), 15:0 iso (about 41%), 15:0 anteiso (about 3%), 15:0 iso 3-OH (about 6.5%), 16:0 3-OH (about 4%) and 17:0 iso 3-OH (about 6.5%). Strains have been isolated as pure cultures under microaerobic conditions from samples of the respiratory tract (lungs, airsac fibrin, pericard) and brain of ducks and geese, always associated with signs similar to those of *R. anatipestifer*-associated exudative septicaemia. In the northern part of Germany, *Coenonia anatina* strains were recovered from over 30% of the cases of septicaemia-related losses in commercial duck flocks in the period 1994–1995. Vaccination with an inactivated whole culture of *Coenonia anatina* strains induced protection of ducklings against *Coenonia anatina*-associated disease. These data suggest its pathogenic role. The DNA G+C content of representative strains varied between 35 and 36 mol%. All *Coenonia anatina* strains listed in Table 1 have been deposited in the BCCM/LMG bacteria collection (URL <http://www.belspo.be/bccm/lmg.htm>). The type strain is LMG 14382^T, which was isolated from a Pekin duck in Germany. Its DNA G+C content is 35 mol%.

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