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# *Ornithobacterium rhinotracheale* gen. nov., sp. nov., Isolated from the Avian Respiratory Tract

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**The phylogenetic position and various genotypic, chemotaxonomic, and classical phenotypic characteristics of 21 gram-negative avian isolates were studied. These strains constitute a genotypically homogeneous taxon in rRNA superfamily V, as shown by DNA-rRNA hybridization data. Determination of the 16S rRNA sequence of this taxon revealed its detailed position within the "flavobacter" subgroup of the "flavobacter-bacteroides" phylum as described by Gherna and Woese (R. Gherna and C. R. Woese, Syst. Appl. Microbiol. 15:513–521, 1992). This new taxon is only distantly related to other members of the "flavobacter-bacteroides" phylum and is therefore given separate generic status. The DNA-DNA binding values for members of this taxon, for which we propose the name *Ornithobacterium rhinotracheale*, confirmed that all of the strains are highly interrelated (DNA-DNA binding values greater than 90% were measured). The G+C contents of members of this taxon are between 37 and 39 mol%. An analysis of the cellular proteins and fatty acids and classical phenotypic characteristics allowed us to distinguish *O. rhinotracheale* from phenotypically similar taxa, such as *Riemerella anatipestifer* and *Capnocytophaga* species. The respiratory quinone content (menaquinone 7) and carbohydrate pattern of *O. rhinotracheale* conform with the respiratory quinone contents and carbohydrate patterns of other members of rRNA superfamily V.**

In the past decade, we determined the protein and fatty acid profiles of a large number of avian isolates which could not be classified after primary identification tests were performed in veterinary laboratories. A total of 21 of these isolates had very similar profiles and differed clearly from all of the other strains investigated, including reference strains of well-known fowl pathogens. These 21 strains were isolated from the respiratory tracts of turkeys (10 strains), chickens (7 strains), rooks, (3 strains), and a partridge (1 strain). Clinical data were not available for all of these strains. However, most of the strains were associated with various respiratory tract infections, including tracheitis, pericarditis, sinusitis, airsacculitis, and pneumonia. Preliminary data on some of the chemotaxonomic characteristics of four strains have been described previously (36).

We studied a wide range of taxonomic parameters, including genotypic, chemotaxonomic, and classical phenotypic parameters, in order to establish the phylogenetic affiliation and to comprehensively describe the new taxon, for which we propose the name *Ornithobacterium rhinotracheale*. Special emphasis was given to the differentiation of *O. rhinotracheale*, *Riemerella anatipestifer*, and *Capnocytophaga* species as these taxa share a number of phenotypic characteristics.

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## MATERIALS AND METHODS

**Isolation of *O. rhinotracheale* strains.** Samples were inoculated onto media routinely used in veterinary laboratories, such as blood agar (e.g., Columbia agar base [catalog no. CM331; Oxoid] supplemented with 5 or 7% defibrinated sheep blood) or chocolate agar. The inoculated plates were incubated at different temperatures under various atmospheric conditions.

**Bacterial strains and growth conditions.** *O. rhinotracheale* strains and *Riemerella* and *Capnocytophaga* reference strains were grown on Trypticase soy agar (catalog no. 11768; BBL, Becton Dickinson Microbiology Systems, Cockeysville, Md.) and were incubated at 36 to 37°C in a microaerobic atmosphere containing approximately 5% $O_2$, 3.5% $CO_2$, 7.5% $H_2$, and 84% $N_2$ unless indicated otherwise. The strains used and their sources are shown in Table 1.

Bacteriological purity was checked by plating and examining living cells, using phase-contrast microscopy and Gram-stained cells. For mass cultures, cells were grown in Roux flasks.

**PAGE of whole-cell proteins.** *O. rhinotracheale*, *Riemerella*, and *Capnocytophaga* strains were grown for 48 h on one to three petri dishes. Whole-cell protein extracts were prepared, and sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) was performed as described previously (54).

**Numerical analysis of the protein gel electropherograms.** A densitometric analysis, normalization and interpolation of the protein profiles, and a numerical analysis were performed by using the GelCompar software package (Applied Maths, Kortrijk, Belgium) as described previously (45).

TABLE 1. Designations and sources of *Ornithobacterium*, *Riemerella*, and *Capnocytophaga* strains used

| Strain[a] | Other designations[a] | Depositor[a,b] | Source (place and/or year of isolation) |
|---|---|---|---|
| *O. rhinotracheale* LMG 9085 | CCUG 23170, MCCM 01773 | Baxter-Jones | Turkey, respiratory tract infection (United Kingdom) |
| *O. rhinotracheale* LMG 9086$^T$ | CCUG 23171$^T$, MCCM 01774$^T$ | Baxter-Jones | Turkey, respiratory tract infection (United Kingdom) |
| *O. rhinotracheale* LMG 9087 | CCUG 23172, MCCM 01778 | Baxter-Jones | Turkey, respiratory tract infection (United Kingdom) |
| *O. rhinotracheale* LMG 9088 | CCUG 24022, MCCM 01779 | Bissuel | Turkey, respiratory tract (Plumeliau, France, 1988) |
| *O. rhinotracheale* LMG 10958 | Hommez 4-9, CCUG 30115, MCCM 01780 | | Turkey, respiratory tract (Belgium) |
| *O. rhinotracheale* LMG 10960 | Hommez 4-11, CCUG 30116, MCCM 01784 | | Turkey, trachea (Belgium, 1988) |
| *O. rhinotracheale* LMG 10961 | Hommez 4-12, CCUG 30117, MCCM 01793 | | Turkey, airsac and trachea (Belgium, 1989) |
| *O. rhinotracheale* LMG 10967 | Hommez 5-28-2, CCUG 30118, MCCM 01794 | | Chicken, airsac (Belgium, 1990) |
| *O. rhinotracheale* LMG 10968 | Hommez 5-28-3, CCUG 30119, MCCM 01795 | | Chicken, airsac and liver (Belgium, 1990) |
| *O. rhinotracheale* LMG 10969 | Hommez 5-BN 1638-3, CCUG 30120, MCCM 01804 | | Chicken, lung (Belgium, 1990) |
| *O. rhinotracheale* LMG 11342 | Hommez 4-10, LMG 10959, CCUG 30121, MCCM 01799 | | Commercially kept partridge, liver, subcutaneous tissue and pericardium (Belgium, 1990) |
| *O. rhinotracheale* LMG 11343 | Hommez 5-28, LMG 10966, CCUG 30122, MCCM 01800 | | Chicken, airsac (Belgium, 1989) |
| *O. rhinotracheale* LMG 11553 | Beichel 112, MCCM 00755, CCUG 30123 | | Rook, respiratory tract (Germany, 1983) |
| *O. rhinotracheale* LMG 11554 | Beichel 113, MCCM 01035, CCUG 30124 | | Rook, respiratory tract (Germany, 1983) |
| *O. rhinotracheale* LMG 11555 | Beichel 114, MCCM 01036, CCUG 30125 | | Rook, respiratory tract (Germany, 1983) |
| *O. rhinotracheale* LMG 11556 | Beichel 118, MCCM 01037, CCUG 30126 | | Turkey, respiratory tract infection (Germany, 1981) |
| *O. rhinotracheale* LMG 12589 | Hafez GGD 1280-91, MCCM 01743, CCUG 30445 | Hafez | Turkey, respiratory tract infection (Stuttgart, Germany, 1991) |
| *O. rhinotracheale* LMG 12590 | du Preez BB 4-3290-1, MCCM 01744, CCUG 30446 | du Preez | Broiler chicken, airsac (Republic of South Africa) |
| *O. rhinotracheale* LMG 12591 | du Preez BB 2-3263-3, MCCM 01747, CCUG 30447 | du Preez | Broiler chicken, airsac (Republic of South Africa) |
| *O. rhinotracheale* LMG 12599 | Hafez GGD 1261-91, MCCM 01767, CCUG 30448 | Hafez | Turkey, respiratory tract infection (Stuttgart, Germany, 1991) |
| *O. rhinotracheale* LMG 12600 | du Preez BG 4-7, MCCM 01766, CCUG 30449 | du Preez | Broiler, airsac (Republic of South Africa) |
| *Capnocytophaga ochracea* LMG 11546 | CCUG 15407, ATCC 33596 | ATCC | Periodontitis (Boston, Mass., 1978) |
| *Capnocytophaga sputigena* LMG 11518$^T$ | CCUG 9714$^T$, ATCC 33612$^T$, VPI 12913$^T$ | VPI | Periodontitis (1978) |
| *Capnocytophaga gingivalis* LMG 11514$^T$ | CCUG 9715$^T$, ATCC 33624$^T$, VPI 12914$^T$ | VPI | Periodontitis (Boston, Mass., 1978) |
| *Capnocytophaga canimorsus* LMG 11541 | MCCM 00060, ATCC 35978, CDC C8936, CCUG 24741 | CDC | Human, blood after dog bite (California) |
| *Capnocytophaga cynodegmi* LMG 11513$^T$ | CCUG 24742$^T$, ATCC 49044$^T$, MCCM 00247$^T$ | CDC | Dog, mouth (Virginia, 1979) |
| *R. anatipestifer* LMG 11054$^T$ | MCCM 00568$^T$, CCUG 21370$^T$, ATCC 11845$^T$ | Sommer | Duck, blood |
| *R. anatipestifer* LMG 11056 | CCUG 25000, HPRS 1795 | Harry | Duck (United Kingdom, 1966–1969) |
| *R. anatipestifer* LMG 11057 | CCUG 25001, HPRS 2591 | Harry | Duck (United Kingdom, 1966–1969) |
| *R. anatipestifer* LMG 11059 | CCUG 25005, HPRS 2336 | Harry | Duck (United Kingdom, 1966–1969) |
| *R. anatipestifer* LMG 11060 | CCUG 25054, HPRS 2560 | Harry | Unknown |

[a] ATCC, American Type Culture Collection, Rockville, Md.; Baxter-Jones, C. Baxter-Jones, British United Turkeys, United Kingdom; Bissuel, C. Bissuel, Montalieu-Vercieu, France; CCUG, Culture Collection of the University of Göteborg Department of Clinical Bacteriology, University of Göteborg, Göteborg, Sweden; CDC, Centers for Disease Control and Prevention, Atlanta, Ga.; du Preez, J. H. du Preez, Festive Farms, Olifantifontein, Republic of South Africa; Hafez, H. M. Hafez, Staatliches Tierärztliches Untersuchungsamt Stuttgart, Stuttgart, Germany; Harry, E. G. Harry, Houghton Poultry Research Station, Houghton, United Kingdom; LMG, Culture Collection of the Laboratorium voor Microbiologie, University of Ghent, Ghent, Belgium; MCCM, Medical Culture Collection of Microorganisms, Marburg, Germany; Sommer, A. I. Sommer, Tromsö, Norway; VPI, Virginia Polytechnic Institute and State University, Blacksburg.

[b] Our isolate unless indicated otherwise.

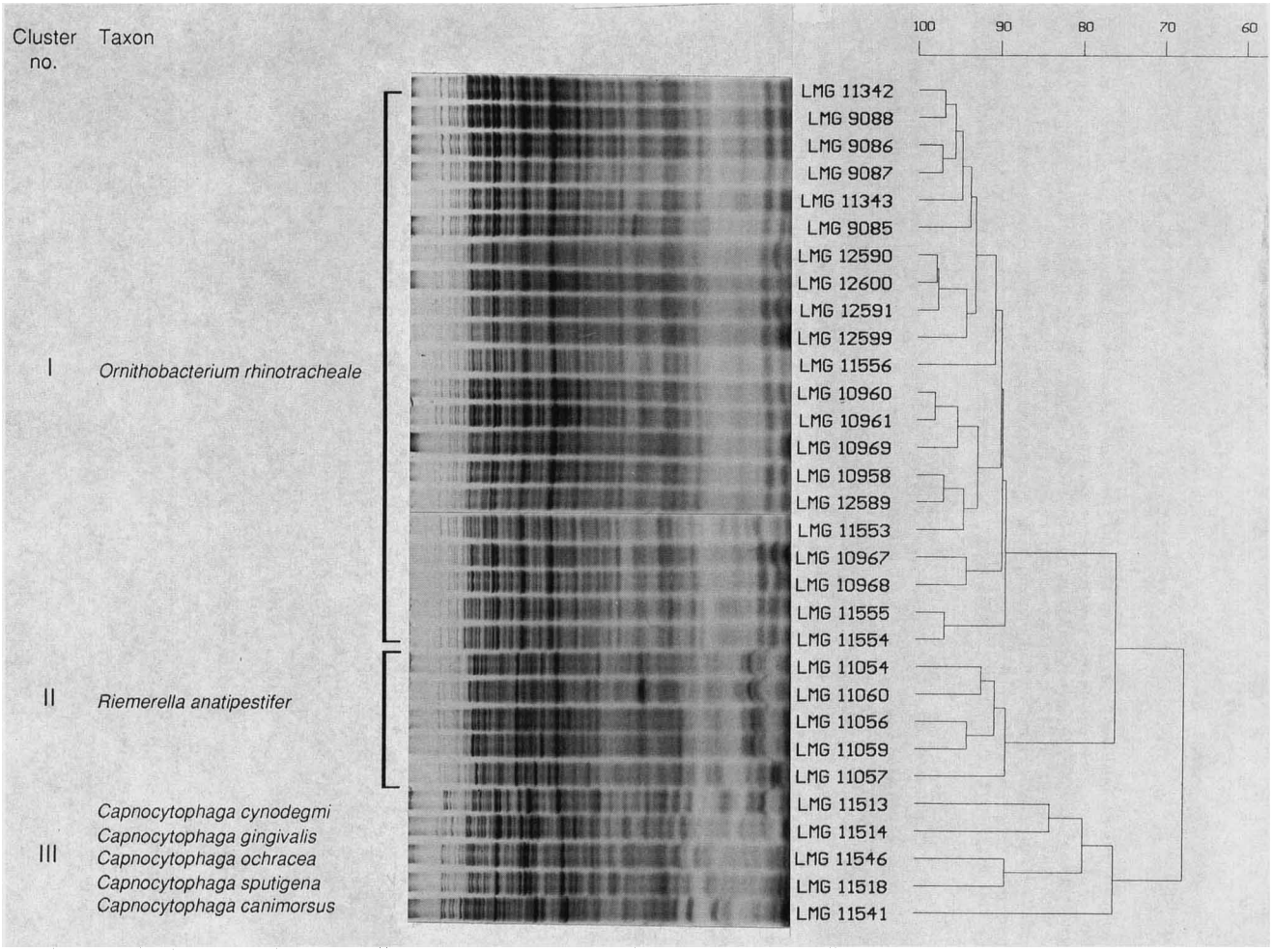


FIG. 1. Protein profiles of *Ornithobacterium*, *Riemerella*, and *Capnocytophaga* strains and corresponding dendrogram derived from unweighed pair group average linkage of correlation coefficients (expressed for convenience as percentages).

**Fatty acid methyl ester analysis.** After incubation for 48 h, a loopful of well-grown cells was harvested, and fatty acid methyl esters were prepared, separated, and identified by using the Microbial Identification System (Microbial ID, Inc., Newark, Del.) as described previously (52).

**Preparation of high-molecular-weight DNA.** High-molecular-weight native DNA was prepared as described previously (52).

**DNA base compositions.** All of the G+C contents were determined by the thermal denaturation method and were calculated by using the equation of Marmur and Doty (33), as modified by De Ley (10).

**DNA-DNA hybridization experiments.** The levels of DNA-DNA binding, expressed as percentages, were determined spectrophotometrically by using the initial renaturation rate method of De Ley et al. (12). Each value given below is the average of the values from at least two hybridization experiments. DNA binding values of 30% or less indicate that there was no significant DNA homology. The total DNA concentration was about 35 μg/ml, and the optimal renaturation temperature in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate [pH 7]) was 66.4°C.

**DNA-rRNA hybridization experiments.** In vivo radioactively labelled rRNA from *O. rhinotracheale* LMG 9086$^T$ (T = type strain) was prepared by using a modification of the procedure of Aiba et al. (1). Bacterial cells were cultivated as described by De Ley and De Smedt (13). The cell growth from a 100-ml broth culture (approximately 1 g) was suspended in 9.5 ml of buffer A containing 0.02 M sodium acetate and 1 mM EDTA (pH 5.5). The cells were lysed by adding 0.5 ml of 10% (wt/vol) SDS. After 10 ml of phenol equilibrated in 0.02 M sodium acetate (pH 5.5) was added, the mixture was incubated for 5 min at 60°C with gentle shaking. The solution was chilled on ice and centrifuged for 10 min at 10,000 rpm. The aqueous phase was reextracted with phenol, and the centrifugation step was repeated. The rRNA was precipitated by adding 2 to 3 volumes of cold (−18°C) ethanol, centrifuged for 5 min at 5,000 rpm, and redissolved in 5 ml of buffer A. The ethanol precipitation step was repeated twice. Finally, the crude rRNA was dissolved in 1× SSC. All materials and solutions were autoclaved in a pressure cooker.

Radioactively labelled rRNAs from reference strains were obtained from members of the Ghent research group.

Purification of rRNA fractions, fixation of single-stranded DNA on membrane filters, chemical determination of the amount of DNA on a filter, saturation hybridization, RNase treatment, and measurements of the thermostabilities of the hybrids were performed as described by Van Landschoot and De Ley (53). Each DNA-rRNA hybrid was character-

TABLE 2. Fatty acid compositions of the strains studied

| Fatty acid | % in[a]: | | |
|---|---|---|---|
| | *O. rhinotracheale* (21 strains) | *R. anatipestifer* (5 strains) | *Capnocytophaga* spp. (5 strains) |
| 13:0 iso | Tr (6) | 15.3 ± 4.8 (5) | 2.1 ± 1.0 (5) |
| ECL 13.566[b] | 4.2 ± 1.5 (21) | 1.2 ± 0.4 (5) | 1.9 ± 0.4 (5) |
| 14:0 | Tr (15) | ND | 1.3 ± 0.4 (5) |
| 15:0 iso | 57.4 ± 6.1 (21) | 52.2 ± 2.5 (5) | 67.4 ± 2.6 (5) |
| 15:0 anteiso | Tr (7) | 5.6 ± 1.4 (5) | 1.5 ± 0.4 (5) |
| 16:0 | 2.9 ± 1.3 (21) | Tr (2) | 2.2 ± 0.4 (5) |
| 15:0 iso 3OH | 8.1 ± 1.9 (21) | 8.1 ± 2.7 (5) | 3.2 ± 1.5 (5) |
| ECL 16.580 | 1.1 ± 0.5 (18) | Tr (3) | 1.0 ± 0.3 (5) |
| 17:0 iso | 1.5 ± 1.0 (19) | ND | Tr (3) |
| 16:0 iso 3OH | ND | 1.1 ± 0.7 (5) | ND |
| 16:0 3OH | 2.8 ± 1.8 (21) | Tr (3) | 4.6 ± 1.3 (5) |
| 17:0 iso 3OH | 20.2 ± 5.0 (21) | 13.9 ± 3.4 (5) | 13.2 ± 2.1 (5) |

[a] The fatty acids for which the average amount for all three taxa was less than 1% are not given; therefore, the sum of the percentages for each group is not 100%. Mean ± standard deviation. The numbers in parentheses are the numbers of strains which contain the fatty acids. Tr, trace (less than 1%); ND, not detected.

[b] ECL, equivalent chain length.

TABLE 3. DNA-DNA hybridization values for *O. rhinotracheale* strains

| Strain | % of DNA binding to strain[a]: | | | |
|---|---|---|---|---|
| | LMG 11556 | LMG 9086$^T$ | LMG 12590 | LMG 10960 |
| LMG 11556 | 100 | | | |
| LMG 9086$^T$ | 93 | 100 | | |
| LMG 12590 | | 100 | 100 | |
| LMG 10960 | | 98 | 98 | 100 |

[a] The values are the averages of the values from at least two hybridization experiments.

ized by the melting temperature of elution [$T_{m(e)}$], the temperature at which 50% of the hybrid was denatured. A homologous duplex was a duplex formed between DNA and rRNA of the same strain; a heterologous hybrid was a duplex formed between DNA and rRNA of different strains. The higher the $T_{m(e)}$ of a heterologous hybrid, the more closely the two strains were related. The $T_{m(e)}$ values from reciprocal hybridization experiments in which all of the strains of each rRNA branch were used to calculate the average linkage level between each pair of rRNA branches.

**16S rRNA sequencing.** rRNA was isolated and partially purified by using a modification of the procedure of Pace et al. (40), as previously described (41). rRNA sequences were determined by using a modification of the standard Sanger dideoxy chain termination technique in which primers complementary to conserved regions were elongated with avian myeloblastosis virus reverse transcriptase (29). In addition to the standard primers (primers 2 through 5, 9, and 10 of Eaton et al. [17]), the following two primers were used: 5′-GGGGTTGCGCTCGTTATAGGACTT-3′ (1093 reverse) and 5′-ACTAGCGATTCCAGCTTC-3′ (1331 reverse).

**Phylogenetic analysis of 16S rRNA sequence information.** The sequences were entered in the RNA program, a program designed for analysis of 16S rRNA data and written in Microsoft QuickBASIC for use with IBM-PC-compatible computers, and were aligned as previously described (41). The data base contained approximately 250 sequences determined in our laboratory (B. P. and F. D.) and 200 sequences obtained from GenBank or from other researchers. Similarity matrices were constructed from aligned sequences by using only those base positions for which 90% of the strains had data. The similarity matrices were corrected for multiple base changes by the method of Jukes and Cantor (24). Phylogenetic trees were constructed by the neighbor-joining method (44).

**Phenotypic tests.** A number of classical phenotypic tests and API ZYM tests were performed in three independent laboratories. Tests for the following characteristics were performed in the laboratory at Klinikum der Philipps-Universität Marburg, Marburg, Germany: motility; growth condition requirements (including medium, temperature, and atmospheric requirements); cell and colony morphology; presence of oxidase, catalase (as described by Kilian [28]), alkaline phosphatase, arginine dihydrolase, lysine and ornithine decarboxylase, β-galactosidase (*o*-nitrophenyl-β-D-galactopyranoside test), and urease activities (as described by Lautrop [30]); growth on MacConkey agar, Endo agar, and Simmons citrate medium; reduction of nitrate; production of indole and hydrogen sulfide; hydrolysis of esculin; oxidation of ethanol; action on D-glucose in oxidation-fermentation medium; and acidification of wheat starch. All tests were performed as described by Mannheim et al. (32) unless indicated otherwise.

The following characteristics were examined in the laboratory at the Department of Clinical Bacteriology, University of Göteborg, Göteborg, Sweden, as described by De Vos et al. (14): cell and colony morphology; growth condition requirements (including medium, temperature, and atmospheric requirements); hemolysis and odor on horse blood agar; formation of pigment on nutrient agar; growth on Drigalski agar; motility; presence of oxidase, catalase, β-galactosidase (*o*-nitrophenyl-β-D-galactopyranoside test), urease, lysine and ornithine decarboxylase, and arginine dihydrolase activities; formation of indole; reactions in triple sugar iron agar; reduction of nitrate and nitrite; denitrification; action on D-glucose in oxidation-fermentation medium; resistance to penicillin (10 μg per disc); liquefaction of gelatin; requirement of X or V factor or porphyrin; and growth with D-galactose, D-glucose, lactose, D-mannitol, D-mannose, D-sorbitol, sucrose, and D-xylose as sole carbon sources.

Tests for the following characteristics were performed with 14 *O. rhinotracheale* strains (they were not performed with strains LMG 11553, LMG 11554, LMG 11555, LMG 11556, LMG 12589, LMG 12590, and LMG 12591) in the laboratory at the Klinik für Geflügel der Tierärztlichen Hochschule, Hannover, Germany: motility; growth condition requirements; cell and colony morphology; presence of oxidase (26), catalase (27), lecithinase (on lecithinase agar [catalog no. SR47; Oxoid]), DNase (on DNase test agar [catalog no. 10449; Merck]), and urease (30) activities; growth on MacConkey agar (catalog no. 5465; Merck) and Drigalski agar (catalog no. 5316; Merck); esculin hydrolysis in esculin broth (catalog no. 3862; Merck); phenylalanine deaminase activity and use of malonate as a carbon source in malonate phenylalanine broth (catalog no. 5419; Merck); nitrate reduction in nitrate broth (catalog no. 10234; Merck); indole production in standard II nutrient broth (catalog no. 7884; Merck); β-galactosidase activity (*o*-nitrophenyl-β-D-galactopyranoside test); ornithine and lysine decarboxylase and arginine dihydrolase activities (34); gelatin hydrolysis (19); hyaluronidase (25) and chondroitin sulfatase (46) activities; production of acetylmethylcarbinol and the methyl red

TABLE 4. $T_{m(e)}$ values for DNA-rRNA hybrids

| Organism used for DNA isolation | $T_{m(e)}$ (°C) of hybrid with rRNA from: | | | | | |
|---|---|---|---|---|---|---|
| | *O. rhinotracheale* LMG 9086$^T$ | *Capnocytophaga ochracea* LMG 11546 | *R. anatipestifer* LMG 11054$^T$ | *Flavobacterium aquatile* LMG 4008$^T$ | *Flavobacterium breve* LMG 4011$^T$ | *Flavobacterium indologenes* LMG 8337$^T$ |
| *O. rhinotracheale* LMG 9085 | 76.8 | 63.7 | | | | |
| *O. rhinotracheale* LMG 9086$^T$ | 75.9 | 62.9 | 66.5 | 63.3 | 65.9 | 64.7 |
| *O. rhinotracheale* LMG 11553 | 76.1 | | | | | |
| *O. rhinotracheale* LMG 11556 | 77.3 | | | | | |
| *Capnocytophaga ochracea* LMG 11546 | 61.9 | 75.7 | 61.8 | 63.7 | 62.0 | 62.0 |
| *Capnocytophaga sputigena* LMG 11518$^T$ | | 74.7 | | 68.4 | | |
| *Capnocytophaga gingivalis* LMG 11514$^T$ | | 70.3 | | 66.3 | 61.2 | |
| *R. anatipestifer* LMG 11054$^T$ | 67.2 | 64.5 | 77.5 | 65.0 | 64.5 | 72.4 |
| *R. anatipestifer* LMG 11602 | 67.5 | | 77.6 | | 72.6 | 72.6 |
| *Flavobacterium aquatile* LMG 4008$^T$ | 63.9 | 65.9 | | 77.0 | 64.7 | 63.5 |
| *Flavobacterium breve* LMG 4011$^T$ | 65.0 | 63.9 | 64.0 | 64.6 | 76.4 | 63.9 |
| *Flavobacterium indologenes* LMG 8337$^T$ | | 62.8 | 71.7 | 65.1 | 66.4 | 78.1 |
| *Flavobacterium meningosepticum* LMG 12279$^T$ | 66.1 | 64.5 | 72.7 | 65.2 | 67.2 | 72.2 |

test (7); and acid production from carbohydrates in phenol red broth base containing 1% carbohydrate with and without chicken serum.

API ZYM tests were performed in all three laboratories according to the recommendations of the manufacturer (bioMérieux, La Balme-les-Grottes, Montalieu-Vercieu, France).

The presence of the following enzymes or enzymatic activities was evaluated by using experimental API galleries (these characteristics were examined only by workers in the Department of Clinical Bacteriology, University of Göteborg, Göteborg, Sweden): production of acid from glucose, fructose, lactose, maltose, sucrose, D-glucosaminic acid (test not performed with strains LMG 12589, LMG 12590, LMG 12591, LMG 12599, and LMG 12600), D-saccharic acid (test not performed with strains LMG 12589, LMG 12590, LMG 12591, LMG 12599, and LMG 12600), dextrin, L-fucose (test not performed with strains LMG 12589, LMG 12590, LMG 12591, LMG 12599, and LMG 12600) galactose, *N*-acetyl-glucosamine, lactulose, mannitol, mannose, ribose, sorbitol, trehalose, and xylose (test not performed with strains LMG 12589, LMG 12590, LMG 12591, LMG 12599, and LMG 12600); penicillinase; phenylalanine deaminase; alanine arylamidase; gamma-glutamyl arylamidase; glycine arylamidase; hydroxyproline arylamidase; lysine arylamidase; proline arylamidase; pyroglutamic acid arylamidase; α-glutamyl-α-glutamic acid arylamidase; glycyl-phenylalanine arylamidase; phenylalanyl-arginine arylamidase; prolyl-arginine arylamidase; seryl-methionine arylamidase; 2-glycyl-glycyl-arginine arylamidase; alanyl-phenylalanyl-prolyl-alanine arylamidase; hippurate hydrolysis; phospholipase (test not performed with strains LMG 12589, LMG 12590, LMG 12591, LMG 12599, and LMG 12600); and phosphodiesterase.

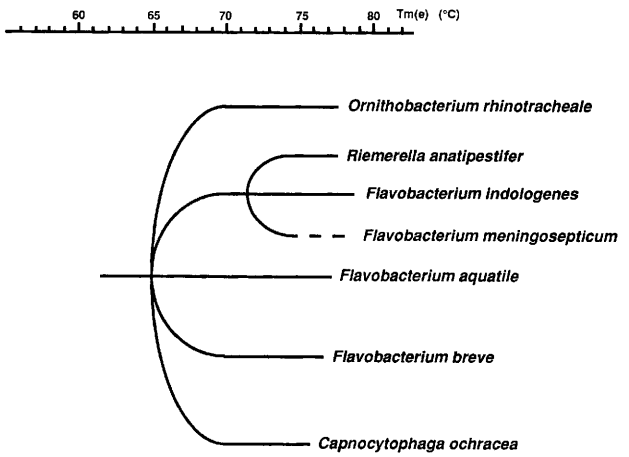


FIG. 2. Simplified rRNA cistron similarity dendrogram of part of rRNA superfamily V.

**Carbohydrate analysis.** The cellular carbohydrates of all *O. rhinotracheale* strains except LMG 10958 and LMG 12590 were analyzed as described previously (3, 37). A loopful of well-grown cells was hydrolyzed in 2 N hydrochloric acid at 100°C for 20 min. Lipids were removed by hexane extraction, and the carbohydrates remaining in the dry residue of the aqueous phase were transformed to the corresponding peracetylated aldononitriles, peracetylated *o*-methyloximes, or alditol acetates. The peracetylated sugars were purified and subsequently analyzed by capillary gas chromatography and mass spectrometry.

## RESULTS

**Isolation of *O. rhinotracheale* strains.** *O. rhinotracheale* can be isolated on common nonselective blood or chocolate agar media from samples obtained from the nasal cavity and infraorbital sinus, but large numbers of other bacteria may mask or overgrow the pinpoint colonies. However, in birds exhibiting pathological lesions of the lower respiratory tract, the organism can be found in pure culture in specimens from the trachea, lung, or airsac exudate. Inoculated plates should be incubated for at least 48 h at 37°C under microaerobic conditions (candle jar) or in air enriched with 10% $CO_2$; 2-day-old colonies are circular, small (approximately 1 mm in diameter), opaque to grayish, and butyrous.

**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 level of correlation between duplicate protein patterns was ≥0.95.

Figure 1 shows the results of a numerical comparison of the protein profiles of all of the *O. rhinotracheale*, *Capnocytophaga*, and *R. anatipestifer* strains investigated. Cluster I contains all 21 isolates of *O. rhinotracheale*, which clus-

TABLE 5. Organisms used for 16S rRNA sequence comparisons and their culture collection numbers, nucleotide sequence accession numbers, and references

| Strain[a] | Nucleotide sequence accession no.[b] | Reference |
|---|---|---|
| *Ornithobacterium rhinotracheale* LMG 9086$^T$ | L19156 | This study |
| *Capnocytophaga canimorsus* ATCC 35979$^T$ | L14637 | This study |
| *Capnocytophaga cynodegmi* ATCC 49044$^T$ (= LMG 11513$^T$) | L14638 | This study |
| *Capnocytophaga gingivalis* ATCC 33624$^T$ (= LMG 11514$^T$) | L14639 | This study |
| *Capnocytophaga ochracea* ATCC 33596 (= LMG 11546) | L14635 | This study |
| *Capnocytophaga sputigena* ATCC 33612$^T$ (= LMG 11518$^T$) | L14636 | This study |
| *Cytophaga aquatilis* ATCC 29551$^T$ | M58764 | 20 |
| *Cytophaga flevensis* ATCC 27944$^T$ | M58767 | 20 |
| *Cytophaga johnsonae* ATCC 17061$^T$ | M59051 | 20 |
| *Cytophaga johnsonae* DSM 425 | M59053 | 20 |
| *Cytophaga latercula* ATCC 23177$^T$ | M58769 | 20 |
| *Cytophaga lytica* ATCC 23178$^T$ | M28058 | 58 |
| *Cytophaga marinoflava* ATCC 19326$^T$ | M58770 | 20 |
| *Cytophaga uliginosa* ATCC 14397$^T$ | M28238 | 58 |
| *Flavobacterium aquatile* ATCC 11947$^T$ | M28236 | 58 |
| *Flavobacterium balustinum* ATCC 33487$^T$ | M58771 | 20 |
| *Flavobacterium breve* ATCC 14234 | M59052 | 20 |
| *Flavobacterium gleum* ATCC 35910$^T$ | M58772 | 20 |
| *Flavobacterium gondwanese* DSM 5423 | M92278 | 16 |
| *Flavobacterium indologenes* ATCC 29897$^T$ | M58773 | 20 |
| *Flavobacterium indoltheticum* ATCC 27950$^T$ | M58774 | 20 |
| *Flavobacterium meningosepticum* ATCC 13253$^T$ | M58776 | 20 |
| *Flavobacterium odoratum* ATCC 4651$^T$ | M58777 | 20 |
| *Flavobacterium salegens* DSM 5424 | M92279 | 16 |
| *Flectobacillus glomeratus* ATCC 43844$^T$ | M58775 | 20 |
| *Flexibacter aurantiacus* ATCC 31107$^T$ | M28054 | 58 |
| *Flexibacter aggregans* ATCC 23162$^T$ | M58780 | 16 |
| *Flexibacter columnaris* ATCC 43622 | M58781 | 20 |
| *Sporocytophaga cauliformis* DSM 3657 | M93151 | 20 |
| *Vesiculata antartica* ATCC 49675 | M61002 | 23 |
| *Weeksella virosa* ATCC 43766$^T$ | M93152 | 20 |
| *Weeksella zoohelcum* ATCC 43767$^T$ | M93153 | 20 |

[a] Abbreviations: DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany; for other abbreviations, see Table 1, footnote *a*.

[b] 16S rRNA sequences are available for electronic retrieval from the GenBank and EMBL data bases under the accession numbers indicated.

tered together at a similarity level of more than 89%. Cluster II consists of five *R. anatipestifer* reference strains that clustered together at a similarity level of more than 89%. Cluster III contains five *Capnocytophaga* reference strains, which clustered together at a similarity level of more than 76%.

**Fatty acid methyl ester composition.** The average fatty acid methyl ester compositions of the *O. rhinotracheale*, *Riemerella*, and *Capnocytophaga* strains examined are shown in Table 2. Fatty acids 15:0 iso, 15:0 iso 3OH, and 17:0 iso 3OH are the predominant fatty acids in all of the strains studied. The high percentages of 17:0 iso 3OH and an unidentified fatty acid with an equivalent chain length of 13.566 differentiate *O. rhinotracheale* from *Capnocytophaga* spp. and *R. anatipestifer*. The latter taxon is characterized by two additional fatty acids, 13:0 iso and 15:0 anteiso, which are present to much lesser extents in the other taxa. Additional characteristics that differentiate *Capnocytophaga* spp. from the other taxa are the relatively low percentage of 15:0 iso 3OH and the relatively high percentage of 16:0 3OH. As shown by the small standard deviations, no significant differences were noted among the reference strains of the five *Capnocytophaga* species (data not shown).

**DNA base compositions.** Four *O. rhinotracheale* strains (LMG 9085, LMG 9086$^T$, LMG 11553, and LMG 11556) were selected at random to determine DNA base ratios; the base compositions of these strains were 39, 38, 37, and 38 mol% G+C, respectively.

**DNA-DNA hybridization results.** Four *O. rhinotracheale* strains (LMG 9086$^T$, LMG 10960, LMG 11556, and LMG 12590) were selected at random for DNA-DNA hybridization experiments. DNA binding values greater than 93% were obtained (Table 3).

**DNA-rRNA hybridization results.** The DNA-rRNA hybridization results are shown in Table 4 (all values for cross-reactions between reference strains will be published elsewhere [45]) and are presented as a simplified dendrogram based on the $T_{m(e)}$ values of the hybrids in Fig. 2. The position of *O. rhinotracheale* within the *Flavobacterium-Cytophaga* rRNA complex (rRNA superfamily V) is shown in Fig. 2. The average $T_{m(e)}$ values obtained from about 80 reciprocal hybridizations between strains belonging to different rRNA branches were used to calculate the average linkage level between each pair of rRNA branches (Table 4) (45). This linkage level is at an average $T_{m(e)}$ of 65.1 ± 1.5°C. The detailed structure of rRNA superfamily V is discussed elsewhere (45). The genera *Capnocytophaga* and *Riemerella* occupy separate positions and branch off at the same base level.

**16S rRNA sequence analysis.** The 16S rRNA sequences of *O. rhinotracheale* LMG 9086$^T$ and five *Capnocytophaga* species have been deposited in the GenBank data base and are available for electronic retrieval under the accession numbers shown in Table 5. The accession numbers of additional reference organisms included in the 16S rRNA phylogenetic analysis are also shown in Table 5.

The results of a comparison between the 16S rRNA sequence of strain LMG 9086$^T$ and the sequences of 31 reference species are shown in Table 6. The matrix is based on comparisons at 1,422 base positions for which more than 90% of the strains had data. The lower half of the matrix is expressed as percentages of differences corrected for multiple base changes by the method of Jukes and Cantor (24). A phylogenetic tree determined from the corrected matrix by using the neighbor-joining method is shown in Fig. 3. Species representing the *Cytophaga* and "flavobacter" subgroups of the "flavobacter-bacteroides" phylum are shown.

**Phenotypic analysis.** The results of classical phenotypic tests and reactions in API ZYM galleries were determined for only *O. rhinotracheale* strains. Similar results were found for nearly all of the tests performed in three independent laboratories, regardless of the method used; however, a number of discrepancies were observed. All of the strains exhibited urease activity if the Lautrop nonproliferative test (30) was used, whereas only 13 of 21 strains produced urease in Christensen medium without agar. All strains produced oxidase activity when the method of Mannheim et al. (32) and Kersters and De Ley (26) was used, whereas only 15 of 21 strains produced oxidase when the Pathotec system was used and only 17 of 21 strains produced oxidase when

TABLE 6. Similarity matrix based on 16S rRNA sequence comparisons

| | % Similarity and % difference compared with[a]: | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Orh* | *Fme* | *Wzo* | *Fgl* | *Fig* | *Fin* | *Fba* | *Fbr* | *Wvi* | *Coc* | *Csp* | *Ccy* | *Cca* |
| *Ornithobacterium rhinotracheale* | — | 89.9 | 86.6 | 86.9 | 86.9 | 87.4 | 87.7 | 89.9 | 88.1 | 84.8 | 84.2 | 84.2 | 84.1 |
| *Flavobacterium meningosepticum* | 10.8 | — | 92.6 | 94.2 | 94.2 | 94.3 | 93.9 | 91.4 | 89.6 | 85.6 | 85.0 | 86.2 | 85.9 |
| *Weeksella zoohelcum* | 14.8 | 7.8 | — | 95.2 | 94.9 | 92.8 | 93.1 | 86.6 | 86.4 | 83.2 | 82.4 | 83.7 | 84.1 |
| *Flavobacterium gleum* | 14.5 | 6.0 | 5.0 | — | 98.9 | 95.6 | 96.1 | 87.2 | 87.1 | 83.0 | 81.9 | 84.1 | 84.0 |
| *Flavobacterium indologenes* | 14.4 | 6.1 | 5.3 | 1.1 | — | 95.4 | 96.3 | 87.1 | 87.2 | 83.1 | 82.0 | 84.5 | 84.1 |
| *Flavobacterium indoltheticum* | 13.8 | 5.9 | 7.6 | 4.5 | 4.8 | — | 97.6 | 87.3 | 87.6 | 82.6 | 81.6 | 83.7 | 83.7 |
| *Flavobacterium balustinum* | 13.4 | 6.3 | 7.2 | 4.1 | 3.8 | 2.5 | — | 88.0 | 87.6 | 82.4 | 81.5 | 83.5 | 83.4 |
| *Flavobacterium breve* | 10.9 | 9.1 | 14.7 | 14.1 | 14.2 | 13.9 | 13.1 | — | 92.8 | 84.7 | 84.2 | 85.2 | 85.2 |
| *Weeksella virosa* | 13.0 | 11.2 | 15.0 | 14.2 | 14.0 | 13.6 | 13.6 | 7.6 | — | 84.9 | 83.7 | 84.9 | 84.8 |
| *Capnocytophaga ochracea* | 17.0 | 15.9 | 19.0 | 19.3 | 19.1 | 19.9 | 20.0 | 17.1 | 16.8 | — | 96.4 | 93.2 | 92.6 |
| *Capnocytophaga sputigena* | 17.7 | 16.8 | 20.0 | 20.8 | 20.5 | 21.2 | 21.2 | 17.8 | 18.4 | 3.7 | — | 93.3 | 92.4 |
| *Capnocytophaga cynodegmi* | 17.7 | 15.2 | 18.4 | 17.9 | 17.4 | 18.4 | 18.6 | 16.4 | 16.9 | 7.1 | 7.1 | — | 98.6 |
| *Capnocytophaga canimorus* | 17.8 | 15.7 | 17.9 | 18.0 | 17.9 | 18.4 | 18.8 | 16.5 | 16.9 | 7.8 | 8.0 | 1.4 | — |
| *Capnocytophaga gingivalis* | 16.6 | 16.2 | 18.4 | 18.5 | 18.2 | 19.6 | 19.3 | 17.7 | 18.0 | 8.9 | 8.8 | 8.4 | 8.9 |
| *Cytophaga uliginosa* | 18.1 | 17.6 | 18.7 | 20.7 | 20.5 | 21.3 | 20.9 | 18.1 | 18.5 | 14.9 | 15.6 | 14.4 | 14.1 |
| *Cytophaga lytica* | 18.2 | 16.1 | 19.2 | 20.6 | 20.0 | 19.5 | 20.1 | 16.3 | 17.7 | 13.5 | 13.7 | 12.9 | 12.1 |
| *Cytophaga marinoflava* | 16.3 | 15.9 | 18.2 | 19.7 | 19.8 | 20.3 | 20.4 | 16.3 | 17.4 | 14.0 | 15.4 | 14.7 | 14.3 |
| *Cytophaga latercula* | 17.8 | 16.3 | 19.9 | 18.8 | 18.9 | 18.0 | 18.7 | 16.1 | 16.3 | 14.2 | 15.2 | 13.0 | 12.6 |
| *Flavobacterium gondwanense* | 16.7 | 16.5 | 19.9 | 21.3 | 21.0 | 20.5 | 20.5 | 16.5 | 17.8 | 14.9 | 16.0 | 14.3 | 13.9 |
| *Flavobacterium salegens* | 17.8 | 17.2 | 20.2 | 21.5 | 21.5 | 20.6 | 20.8 | 17.3 | 17.8 | 15.6 | 16.7 | 16.0 | 15.3 |
| *Flectobacillus glomeratus* | 17.4 | 16.6 | 18.7 | 19.5 | 19.3 | 18.4 | 18.2 | 17.4 | 16.8 | 15.7 | 16.1 | 15.9 | 14.8 |
| *Vesiculata antarctica* | 16.7 | 15.6 | 18.8 | 19.6 | 19.7 | 17.9 | 17.9 | 16.7 | 17.2 | 15.9 | 16.3 | 16.4 | 15.4 |
| *Flexibacter aggregans* | 17.6 | 15.7 | 19.3 | 19.5 | 19.3 | 19.3 | 18.8 | 18.1 | 17.7 | 15.6 | 16.0 | 15.2 | 14.2 |
| *Flavobacterium odoratum* | 15.9 | 14.8 | 18.7 | 18.1 | 18.0 | 17.9 | 16.8 | 15.2 | 14.5 | 14.4 | 15.1 | 14.7 | 14.6 |
| *Flavobacterium aquatile* | 15.0 | 15.4 | 18.9 | 18.6 | 18.8 | 17.1 | 17.3 | 14.8 | 15.6 | 13.2 | 13.6 | 12.4 | 11.9 |
| *Cytophaga johnsonae* ATCC 17061$^T$ | 16.6 | 15.7 | 19.4 | 18.3 | 18.3 | 18.6 | 18.3 | 14.6 | 15.0 | 13.2 | 12.6 | 11.0 | 10.9 |
| *Flexibacter aurantiacus* | 16.8 | 15.8 | 19.6 | 18.6 | 18.5 | 18.7 | 18.3 | 14.8 | 15.3 | 13.6 | 12.9 | 11.1 | 11.0 |
| *Cytophaga flevensis* A-34 | 16.4 | 16.2 | 18.3 | 17.5 | 17.5 | 17.7 | 17.1 | 14.9 | 15.8 | 12.8 | 12.6 | 10.1 | 10.0 |
| *Cytophaga aquatilis* | 16.6 | 16.2 | 19.5 | 18.6 | 18.4 | 18.4 | 17.7 | 14.9 | 15.7 | 13.4 | 13.2 | 11.6 | 11.0 |
| *Flexibacter columnaris* | 16.8 | 16.2 | 19.5 | 19.2 | 18.8 | 19.1 | 18.0 | 14.7 | 15.5 | 13.3 | 13.1 | 11.8 | 11.5 |
| *Cytophaga johnsonae* DSM 425 | 16.4 | 15.9 | 19.6 | 19.4 | 19.6 | 18.3 | 18.0 | 14.4 | 15.8 | 12.9 | 12.6 | 11.8 | 11.6 |
| *Sporocytophaga cauliformis* | 16.7 | 16.2 | 19.9 | 19.3 | 19.4 | 18.4 | 18.1 | 14.4 | 15.3 | 13.2 | 13.2 | 11.9 | 11.8 |

[a] The numbers on the upper right are uncorrected levels of similarity; the numbers on the lower left are levels of difference corrected for multiple base changes by the method of Jukes and Cantor. Abbreviations: *Orh*, *Ornithobacterium rhinotracheale*; *Fme*, *Flavobacterium meningosepticum*; Wzo, *Weeksella zoohelcum*; *Fgl*, *Flavobacterium gleum*; *Fig*, *Flavobacterium indologenes*; *Fin*, *Flavobacterium indoltheticum*; *Fba*, *Flavobacterium balustinum*; *Fbr*, *Flavobacterium breve*; *Wvi*, *Weeksella virosa*; *Coc*, *Capnocytophaga ochracea*; *Csp*, *Capnocytophaga sputigena*; *Ccy*, *Capnocytophaga cynodegmi*; *Cca*, *Capnocytophaga canimorsus*; *Cgi*, *Capnocytophaga gingivalis*; *Cul*, *Cytophaga uliginosa*; *Cly*, *Cytophaga lytica*; *Cma*, *Cytophaga marinoflava*; *Cla*, *Cytophaga latercula*; *Gfp*, *Flavobacterium gondwanense*; *Fsa*, *Flavobacterium salegens*; *Fgl*, *Flectobacillus glomeratus*; *Van*, *Vesiculata antarctica*; *Fag*, *Flexibacter aggregans*; *Fod*, *Flavobacterium odoratum*; *Faq*, *Flavobacterium aquatile*; *Cj1*, *Cytophaga johnsonae* ATCC 17061$^T$; *Fau*, *Flexibacter aurantiacus*; *Cfl*, *Cytophaga flevensis* A-34; *Caq*, *Cytophaga aquatilis*; *Fco*, *Flexibacter columnaris*; *Cj2*, *Cytophaga johnsonae* DSM 425; *Sca*, *Sporocytophaga cauliformis*.

Kovacs' reagent (31) was used. Testing for arginine dihydrolase activity by using a heavy inoculum in Möller's medium yielded 19 positive reactions after 3 days of incubation at 36°C (strains LMG 10958 and LMG 11555 did not react). The results obtained with the API ZYM galleries were reproducible only when a heavy inoculum was used. Differences in test results among the three laboratories were found only for the following tests which yielded strain-dependent results (although all strains exhibited at least weak activity): esterase C4, valine arylamidase, cysteine arylamidase, trypsin, and α- and β-galactosidase. Strain-dependent results were recorded in all three laboratories for chymotrypsin activity. The results of the remaining classical and API ZYM tests are described below in the description of the new taxon.

Enzymes belonging to the experimental galleries were tested for *O. rhinotracheale* strains and for five *Capnocytophaga* and *R. anatipestifer* reference strains (Table 1). The following activities were present in all strains: alanine arylamidase; glycine arylamidase; lysine arylamidase; proline arylamidase; α-glutamyl-α-glutamic acid arylamidase; glycyl-phenylalanine arylamidase; phenylalanyl-arginine arylamidase; prolyl-arginine arylamidase; seryl-methionine arylamidase; 2-glycyl-glycyl-arginine arylamidase; and alanyl-phenylalanyl-prolyl-alanine arylamidase. The following characteristics were negative for all strains: production of acid from D-glucosaminic acid, D-saccharic acid, L-fucose, mannitol, sorbitol, trehalose, and xylose; phenylalanine deaminase activity; hippurate hydrolysis; and phospholipase activity. All of the other test results are shown in Table 7. When the API galleries were used, *O. rhinotracheale* LMG 9086$^T$ did not produce acid from glucose, fructose, maltose, sucrose, or dextrin (Table 7), whereas acid production was observed for the same strain in phenol red broth base containing 1% carbohydrate with and without chicken serum. The latter reaction pattern is more in agreement with the general profile of this species (Table 7).

**Carbohydrate profiles.** All of the strains except LMG 9087 contained high amounts of lyxose (up to 30%). Mannose, glucose (except LMG 11554), and galactose were always present; altrose, sorbose, and heptoses were always absent. Ribose was present in most strains. The detailed carbohy-

TABLE 6—*Continued*

| % Similarity and % difference compared with[a]: | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Cgi* | *Cul* | *Cly* | *Cma* | *Cla* | *Gfp* | *Fsa* | *Fgl* | *Van* | *Fag* | *Fod* | *Faq* | *Cj1* | *Fau* | *Cfl* | *Caq* | *Fco* | *Cj2* | *Sca* |
| 85.1 | 83.9 | 83.8 | 85.4 | 84.2 | 85.0 | 84.2 | 84.5 | 85.1 | 84.3 | 85.6 | 86.4 | 85.1 | 85.0 | 85.2 | 85.1 | 84.9 | 85.3 | 85.1 |
| 85.5 | 84.3 | 85.5 | 85.7 | 85.3 | 85.2 | 84.6 | 85.1 | 85.9 | 85.9 | 86.5 | 86.1 | 85.8 | 85.7 | 85.4 | 85.4 | 85.4 | 85.7 | 85.4 |
| 83.6 | 83.4 | 83.1 | 83.9 | 82.6 | 82.5 | 82.3 | 83.4 | 83.3 | 83.0 | 83.4 | 83.3 | 82.9 | 82.8 | 83.7 | 82.8 | 82.9 | 82.8 | 82.5 |
| 83.6 | 81.9 | 82.0 | 82.7 | 83.4 | 81.4 | 81.3 | 82.8 | 82.7 | 82.9 | 83.9 | 83.5 | 83.7 | 83.5 | 84.4 | 83.5 | 83.0 | 82.9 | 83.0 |
| 83.8 | 82.1 | 82.4 | 82.6 | 83.3 | 81.7 | 81.3 | 83.0 | 82.7 | 83.0 | 84.0 | 83.4 | 83.8 | 83.6 | 84.4 | 83.7 | 83.4 | 82.8 | 82.9 |
| 82.8 | 81.5 | 82.8 | 82.2 | 84.0 | 82.1 | 82.0 | 83.7 | 84.1 | 83.0 | 84.1 | 84.7 | 83.5 | 83.5 | 84.3 | 83.7 | 83.1 | 83.8 | 83.7 |
| 83.0 | 81.7 | 82.4 | 82.1 | 83.5 | 82.1 | 81.8 | 83.8 | 84.1 | 83.3 | 85.0 | 84.6 | 83.8 | 83.8 | 84.7 | 84.3 | 84.0 | 84.0 | 83.9 |
| 84.2 | 83.9 | 85.3 | 85.4 | 85.5 | 85.2 | 84.6 | 84.4 | 85.1 | 84.0 | 86.2 | 86.6 | 86.8 | 86.5 | 86.5 | 86.5 | 86.6 | 86.9 | 86.9 |
| 84.0 | 83.6 | 84.2 | 84.5 | 85.3 | 84.2 | 84.1 | 84.9 | 84.6 | 84.2 | 86.8 | 85.9 | 86.4 | 86.2 | 85.7 | 85.8 | 86.0 | 85.7 | 86.2 |
| 91.6 | 86.5 | 87.7 | 87.3 | 87.0 | 86.5 | 85.9 | 85.9 | 85.7 | 85.9 | 86.9 | 87.9 | 87.9 | 87.6 | 88.2 | 87.8 | 87.8 | 88.2 | 87.9 |
| 91.7 | 85.9 | 87.4 | 86.1 | 86.2 | 85.6 | 85.0 | 85.5 | 85.4 | 85.6 | 86.3 | 87.6 | 88.4 | 88.1 | 88.4 | 87.9 | 87.9 | 88.4 | 87.9 |
| 92.1 | 86.9 | 88.2 | 86.6 | 88.0 | 87.0 | 85.6 | 85.7 | 85.2 | 86.2 | 86.6 | 88.6 | 89.7 | 89.7 | 90.5 | 89.3 | 89.0 | 89.1 | 89.0 |
| 91.6 | 87.2 | 88.9 | 87.0 | 88.4 | 87.3 | 86.2 | 86.6 | 86.1 | 87.1 | 86.7 | 89.0 | 89.8 | 89.8 | 90.6 | 89.7 | 89.3 | 89.3 | 89.1 |
| — | 86.4 | 88.1 | 87.6 | 87.9 | 87.0 | 85.7 | 86.8 | 86.5 | 86.1 | 87.2 | 87.9 | 88.8 | 88.8 | 88.0 | 87.7 | 87.9 | 87.2 | 86.8 |
| 15.0 | — | 90.7 | 89.2 | 87.9 | 88.4 | 86.7 | 87.5 | 86.8 | 87.8 | 86.8 | 87.5 | 86.3 | 86.0 | 87.3 | 86.9 | 87.3 | 87.3 | 86.3 |
| 13.0 | 10.0 | — | 90.2 | 92.4 | 90.3 | 89.4 | 89.1 | 89.0 | 89.3 | 89.4 | 89.7 | 89.1 | 89.1 | 89.1 | 89.7 | 89.5 | 89.5 | 89.0 |
| 13.6 | 11.6 | 10.5 | — | 90.1 | 89.5 | 89.6 | 87.8 | 87.3 | 87.3 | 87.6 | 89.5 | 88.2 | 88.0 | 88.3 | 88.6 | 88.8 | 89.2 | 88.8 |
| 13.2 | 13.2 | 8.0 | 10.6 | — | 91.1 | 92.2 | 89.4 | 89.8 | 88.8 | 88.8 | 89.5 | 88.7 | 88.6 | 88.9 | 89.4 | 89.2 | 89.0 | 89.2 |
| 14.3 | 12.6 | 10.4 | 11.3 | 9.5 | — | 91.6 | 87.8 | 88.2 | 88.1 | 86.2 | 88.4 | 87.9 | 87.8 | 88.3 | 88.2 | 88.3 | 88.2 | 88.3 |
| 15.8 | 14.7 | 11.5 | 11.2 | 8.2 | 8.9 | — | 89.8 | 89.2 | 87.3 | 85.3 | 89.1 | 87.2 | 87.1 | 87.8 | 87.9 | 88.1 | 88.0 | 88.3 |
| 14.5 | 13.7 | 11.7 | 13.3 | 11.4 | 13.3 | 10.9 | — | 97.4 | 93.7 | 88.7 | 89.6 | 88.8 | 88.6 | 88.4 | 88.7 | 88.5 | 88.9 | 88.7 |
| 14.9 | 14.6 | 11.8 | 13.9 | 10.9 | 12.8 | 11.6 | 2.7 | — | 93.2 | 88.2 | 89.6 | 88.8 | 88.6 | 88.0 | 88.9 | 88.7 | 89.2 | 89.1 |
| 15.4 | 13.3 | 11.5 | 13.9 | 12.1 | 12.9 | 13.9 | 6.6 | 7.1 | — | 88.6 | 88.6 | 87.8 | 87.7 | 87.9 | 88.2 | 88.0 | 88.0 | 87.4 |
| 14.0 | 14.5 | 11.4 | 13.6 | 12.2 | 15.2 | 16.3 | 12.2 | 12.9 | 12.4 | — | 90.2 | 89.7 | 89.4 | 90.0 | 90.0 | 90.2 | 90.0 | 89.7 |
| 13.2 | 13.6 | 11.0 | 11.4 | 11.3 | 12.6 | 11.7 | 11.2 | 11.2 | 12.4 | 10.5 | — | 93.9 | 94.2 | 94.5 | 95.3 | 95.4 | 95.1 | 95.1 |
| 12.1 | 15.1 | 11.8 | 12.8 | 12.3 | 13.2 | 14.0 | 12.1 | 12.2 | 13.3 | 11.1 | 6.3 | — | 99.6 | 96.4 | 96.0 | 95.9 | 95.9 | 96.4 |
| 12.2 | 15.5 | 11.8 | 13.1 | 12.4 | 13.4 | 14.2 | 12.3 | 12.4 | 13.5 | 11.4 | 6.1 | 0.4 | — | 96.3 | 95.7 | 95.9 | 95.7 | 96.2 |
| 13.1 | 14.0 | 11.7 | 12.7 | 12.0 | 12.7 | 13.3 | 12.6 | 13.1 | 13.2 | 10.7 | 5.8 | 3.7 | 3.8 | — | 97.1 | 97.3 | 97.2 | 96.7 |
| 13.4 | 14.4 | 11.1 | 12.3 | 11.4 | 12.9 | 13.2 | 12.2 | 12.0 | 12.9 | 10.7 | 4.8 | 4.1 | 4.4 | 3.0 | — | 98.5 | 97.7 | 97.1 |
| 13.2 | 14.0 | 11.3 | 12.1 | 11.7 | 12.7 | 13.0 | 12.5 | 12.3 | 13.1 | 10.5 | 4.8 | 4.2 | 4.2 | 2.8 | 1.5 | — | 98.4 | 97.7 |
| 14.0 | 14.0 | 11.3 | 11.7 | 11.9 | 12.8 | 13.1 | 12.0 | 11.7 | 13.1 | 10.7 | 5.1 | 4.2 | 4.4 | 2.9 | 2.3 | 1.7 | — | 98.4 |
| 14.5 | 15.1 | 11.9 | 12.2 | 11.7 | 12.7 | 12.7 | 12.3 | 11.8 | 13.7 | 11.0 | 5.1 | 3.7 | 3.9 | 3.4 | 3.0 | 2.4 | 1.7 | — |

drate profiles of all of the strains studied are shown in Table 8.

## DISCUSSION

In an attempt to identify a large number of veterinary isolates, numerical comparisons of the whole-cell protein and fatty acid contents were used as a first step in the identification process. A total of 21 gram-negative avian isolates had strikingly similar profiles (Fig. 1 and Table 2) which were clearly different from the profiles of all other organisms present in our data bases. Such very high levels of similarity in protein and fatty acid contents for the most part reflect very close genotypic relatedness as well (38, 50–52, 55). DNA binding studies performed with a representative sample of these 21 isolates confirmed that all of the strains belong to a single genospecies (DNA binding values were greater than 93% [Table 3]). In order to establish the phylogenetic affiliation of this taxon, we performed DNA-rRNA hybridization experiments with four selected strains and reference strains belonging to the six rRNA superfamilies sensu De Ley (49). We also determined the nearly complete 16S rRNA sequence and compared it with about 450 sequences representing the various evolutionary lineages within the *Bacteria* (56).

An extensive DNA-rRNA hybridization analysis revealed the position of this taxon on a separate phylogenetic branch within rRNA superfamily V (Fig. 2). Among other taxa, the genera *Flavobacterium*, *Cytophaga*, *Capnocytophaga*, and *Riemerella* are present in the same taxonomic neighborhood (Fig. 2) (45). However, the nearest neighbors of the new taxon are situated at a difference in $T_{m(e)}$ of about 13°C (Fig. 2). Usually, the $T_{m(e)}$ range within a genus is about 5°C (11). This clearly indicates that the 21 strains belong to a very distinct, new genus. The difference in $T_{m(e)}$ of about 13°C corresponds to the genomic range found within several well-characterized bacterial families (49). At present, proposing a name for this family seems premature as it is generally accepted that the taxonomy of related genera, such as *Flavobacterium* and *Cytophaga*, has to be revised thoroughly. A comparison of the 16S rRNA sequence of strain LMG 9086$^T$ with other bacterial 16S rRNA sequences in our data base confirmed the placement of this organism in the *Flavobacterium-Cytophaga* rRNA cluster. It is well-known that rRNA sequencing is more powerful than DNA-rRNA hybridization for establishing relationships at deep phylogenetic levels (i.e., between families or classes) (57). A detailed scheme of the relationships between the new organism and its closest allies is shown in Fig. 3. Obviously, the new taxon is most closely related to species in the "flavobacter" subgroup of the "flavobacter-bacteroides" phylum as described by Gherna and Woese (20). It falls in the "flavobacter" subgroup and is about equally related to the two clusters of *Flavobacterium* species. *Flavobacterium meningosepticum* and *Flavobacterium breve* are its nearest neighbors and are related at a similarity level of 89.9%. This low

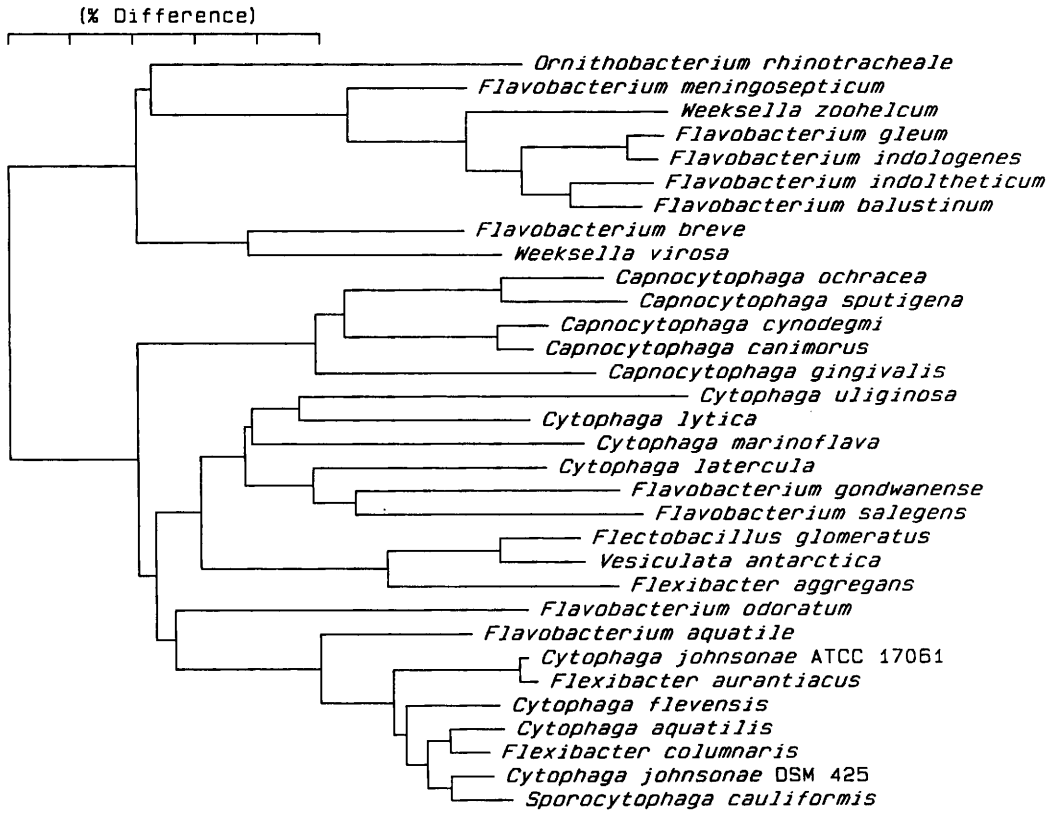


FIG. 3. Phylogenetic tree for *O. rhinotracheale* and 31 reference species based on 16S rRNA sequence similarity values. The scale bar represents 0 to 5% differences in nucleotide sequences as determined by measuring the lengths of the horizontal lines connecting any two species.

similarity level again indicates that there is a large genetic distance between the new taxon and its closest relatives. Also from the viewpoint of direct sequencing, this genetic distance corresponds to the genomic range within several bacterial families (e.g., the family *Cardiobacteriaceae*, whose members cluster at a similarity level of more than 93% [15]). We therefore propose a new genus, *Ornithobacterium*, and a new species, *O. rhinotracheale*, to accommodate this taxon.

**Identification of *O. rhinotracheale* strains.** Typical members of the *Flavobacterium-Cytophaga* rRNA cluster are readily identified on the basis of their flexirubin type pigments. However, strains that produce only weak or no colonial pigments on common isolation media must be recognized in other ways. It has been emphasized previously that chemotaxonomic markers such as respiratory quinones, cellular fatty acids, and cellular carbohydrates are valuable parameters for the identification of these organisms (2, 5, 6, 8, 9, 22, 35, 39, 43, 47, 48). From the viewpoint of veterinary microbiology, it is important to differentiate *O. rhinotracheale* from *R. anatipestifer* as these two taxa share the same ecological niche and have several features in common. In fact, several *O. rhinotracheale* strains were initially identified as *R. anatipestifer* or as *R. anatipestifer*-like (59). Another member of the same rRNA cluster, the genus *Capnocytophaga*, has a similar type of capnophilic metabolism and shares a number of phenotypic features with *O. rhinotracheale* and *R. anatipestifer* (see below). We therefore included a reference strain of each of the five *Capnocytophaga* species and five representative *R. anatipestifer* strains in several phenotypic analyses in order to evaluate the differentiation of the three genera.

All *O. rhinotracheale* and *R. anatipestifer* strains have very similar protein contents ($r > 89\%$) (Fig. 1). More variability was observed in the five *Capnocytophaga* strains. This variability was expected as each strain represents a different species (Table 1 and Fig. 1). In general, a comparison of the protein profiles results in clear-cut differentiation among *O. rhinotracheale*, *R. anatipestifer*, and *Capnocytophaga* species; each of these taxa constitutes a distinct electrophoretic cluster (Fig. 1).

All three taxa are characterized by very high percentages of branched-chain fatty acids (>87%). Similar data for *R. anatipestifer* and *Capnocytophaga* species have been reported previously (2, 9, 22, 39, 43, 45, 47, 48). For each taxon, 15:0 iso accounts for more than one-half of the total fatty acid content (Table 2). The relative amounts of 13:0 iso, 15:0 anteiso, 15:0 iso 3OH, 17:0 iso 3OH, and an unidentified fatty acid with an equivalent chain length of 13.566 can easily be used as differential features (see above). As reported previously (47), strains of different *Capnocytophaga* species have very similar fatty acid compositions (Table 2).

As is common in other members of the *Flavobacterium-Cytophaga* rRNA cluster, menaquinones were the only respiratory quinones detected (5, 8, 18, 47). *O. rhinotracheale* LMG 11553 and LMG 11556 reportedly contain menaquinone 7 as their only respiratory quinones, which is similar to the quinone composition of *R. anatipestifer* (18). *Capnocytophaga* species contain menaquinone 6 and trace amounts of menaquinone 5 (6, 47).

TABLE 7. Differential phenotypic characteristics of the strains studied

| Characteristic | *O. rhinotracheale* | | *R. anatipestifer* (5 strains) | *Capnocytophaga gingivalis* LMG 11514$^T$ | *Capnocytophaga sputigena* LMG 11518$^T$ | *Capnocytophaga ochracea* LMG 11546 | *Capnocytophaga canimorsus* LMG 11541 | *Capnocytophaga cynodegmi* LMG 11513$^T$ |
|---|---|---|---|---|---|---|---|---|
| | Strain LMG 9086$^T$ | No. of strains positive/no. of strains tested[a] | | | | | | |
| Production of acid from: | | | | | | | | |
| Glucose | −[b] | 16/21 | + | + | + | + | + | + |
| Fructose | − | 18/21 (3)[c] | − | − | + | + | − | + |
| Lactose | + | 19/21 (4) | − | − | + | + | + | + |
| Maltose | − | 16/21 | + | w | + | + | + | + |
| Sucrose | − | 2/21 (1) | − | + | + | + | − | + |
| Dextrin | − | 14/21 (1) | +[d] | − | + | + | + | + |
| Galactose | + | 15/16 | − | − | + | + | + | + |
| *N*-acetylglucosamine | + | 15/16 | − | − | + | + | + | + |
| Lactulose | + | 15/16 | − | − | w | + | w | + |
| Mannose | + | 20/21 | + | w | + | + | + | + |
| Ribose | − | 12/21 (4) | − | − | − | − | − | − |
| Penicillin G resistance | − | 14/21 (2) | − | − | − | − | − | − |
| Gamma-glutamyl arylamidase | − | 0/21 | + | + | + | − | − | w |
| Hydroxyproline arylamidase | + | 20/21 (4) | +[e] | + | + | w | w | w |
| Pyroglutamic acid arylamidase | + | 14/21 (2) | +[f] | + | + | + | w | + |
| Phosphodiesterase | + | 16/16 | + | − | + | + | + | + |

[a] The number of positive strains includes the number of strains that exhibited weak activity.
[b] +, positive reaction; −, negative reaction; w, weak reaction.
[c] The numbers in parentheses are the numbers of strains that exhibited weak activity.
[d] Strain LMG 11054$^T$ exhibited weak activity.
[e] Strain LMG 11056 exhibited weak activity.
[f] Strain LMG 11057 exhibited weak activity.

TABLE 8. Carbohydrate profiles of 19 *O. rhinotracheale* strains

| Retention time (min) | Carbohydrate[a] | Peak areas[b] | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | LMG 9085 | LMG 9086[T] | LMG 9087 | LMG 9088 | LMG 10960 | LMG 10961 | LMG 10967 | LMG 10968 | LMG 10969 | LMG 11342 | LMG 11343 | LMG 11553[c] | LMG 11554[c] | LMG 11555 | LMG 11556[c] | LMG 12589 | LMG 12591 | LMG 12599 | LMG 12600 |
| 10.99 | Erythritol | (3) | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 1 | — |
| 11.12 | Fucosylamine (A) (?) | — | — | — | — | — | (4) | — | — | — | 3 | — | — | — | 3 | — | — | — | 1 | (2) |
| 11.44 | $C_5$ | (3) | — | 3 | — | — | — | (3) | — | — | — | — | 4 | 5 | 3 | 4 | 3 | 3 | 3 | 3 |
| 11.67 | $C_5$-$CH_3$ (?) | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 3 | 2 |
| 11.98 | $C_5$ | — | — | — | — | — | — | — | — | — | — | — | 3 | 4 | (3) | 3 | 3 | 2 | 3 | 3 |
| 12.09 | Ribose (A) | 5 | — | 6 | — | 6 | — | 5 | 4 | 4 | 6 | 6 | 6 | 7 | 4 | 4 | (3) | 3 | — | (2) |
| 12.18 | Fucose (A) | 4 | — | 3 | — | — | 4 | — | 5 | 4 | — | — | — | — | 8 | — | (2) | — | 6 | (2) |
| 12.23 | $C_5$ | — | — | — | — | — | — | — | — | — | (3) | — | — | — | — | — | — | — | — | — |
| 12.80 | $C_6$ | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 2 | — | 1 | — |
| 13.29 | Lyxose (A) | 3 | — | — | 4 | 4 | — | 5 | 4 | 4 | 4 | 5 | 4 | 4 | 6 | 4 | 5 | 4 | 5 | 5 |
| 13.34 | Rhamnose (A) | — | — | — | — | — | 2 | — | 5 | — | — | — | — | — | — | — | — | — | — | — |
| 13.50 | Threose (?) | — | — | — | — | — | — | — | — | 2 | — | — | — | — | — | — | — | — | (1) | — |
| 13.56 | Lyxose (O) | 5 | 3 | — | 6 | 6 | 4 | 7 | 6 | 6 | 6 | 6 | 6 | 7 | 7 | 6 | (7) | 6 | 7 | (8) |
| 13.63 | Rhamnose (O) | 3 | — | — | — | — | 4 | — | 7 | 3 | 3 | — | 4 | — | — | — | — | 1 | — | — |
| 13.68 | Fucose (O) | — | — | — | — | — | — | — | — | 4 | 3 | — | — | — | — | — | (3) | — | — | 2 |
| 13.69 | Arabinose (A) | — | — | — | — | — | — | — | — | 4 | — | — | — | — | — | — | (2) | (3) | 2 | — |
| 13.75 | Threose (O) (?) | (3) | (4) | (3) | — | — | — | (3) | — | — | (3) | — | — | — | — | — | — | — | — | (2) |
| 13.91 | Arabinose (O) | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 1 | 2 | 1 | 1 |
| 14.51 | $C_5$-ol (?) | — | — | — | — | (1) | — | — | — | — | — | — | — | — | — | — | 1 | — | 1 | — |
| 15.28 | $C_6$-ol | 4 | — | 5 | — | 4 | — | — | — | (3) | 2 | — | 4 | 3 | — | 4 | 3 | 3 | 3 | 3 |
| 15.32 | $C_6$ (?) | — | — | — | — | (1) | (2) | — | — | (4) | — | — | — | — | — | — | (3) | 2 | 2 | — |
| 15.41 | Mannose (A) | 5 | 5 | 5 | 5 | 4 | 3 | 4 | 4 | 4 | 4 | 3 | 4 | 3 | 5 | 3 | 3 | 4 | 5 | 5 |
| 15.47 | $C_6$-ol (?) | — | — | — | (3) | — | — | — | — | — | — | — | — | — | — | — | (2) | 2 | 1 | 2 |
| 15.54 | Glucose (A) | 3 | 4 | 4 | 5 | 5 | 5 | — | 4 | 4 | 4 | 4 | 4 | — | 5 | 3 | 3 | 4 | 4 | 4 |
| 15.78 | $C_6$-amine | — | — | — | — | — | — | — | — | — | — | — | 2 | — | — | — | — | — | — | — |
| 15.83 | Galactose (A) | 5 | 7 | 5 | 5 | 6 | 4 | 5 | 5 | 4 | 5 | 4 | 4 | 4 | 5 | 2 | 4 | 4 | 6 | 3 |
| 16.32 | Galactose (O) | 4 | 4 | 5 | 3 | 4 | 6 | 5 | 4 | 5 | 6 | 3 | 3 | — | 3 | — | 5 | 4 | 4 | 3 |
| 16.42 | Mannose (O) | 3 | 3 | — | 3 | 3 | 3 | 4 | (3) | 4 | 4 | — | 2 | — | 2 | 3 | 3 | 3 | 3 | 3 |
| 16.60 | Allose (O) | — | 4 | (3) | — | — | 3 | (3) | 3 | 2 | 3 | — | 2 | — | 3 | — | — | 3 | 2 | (3) |
| 16.70 | Mannose (O) | 5 | 6 | 6 | 5 | 6 | 5 | 5 | (5) | 6 | 6 | 5 | 4 | 3 | 3 | 5 | (5) | 5 | 5 | 4 |
| 16.75 | Glucose (O) | 3 | 4 | 4 | 5 | 4 | 5 | 4 | 5 | 4 | 6 | 5 | 4 | — | 4 | 3 | 3 | 4 | 4 | 5 |
| 16.85 | Galactose (O) | 5 | 6 | 6 | 5 | 5 | 7 | 5 | 6 | 7 | 7 | 5 | 5 | 3 | 4 | 3 | 6 | 6 | 6 | 4 |
| 17.01 | Sorbose (O) | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 1 | — | — | — |
| 17.14 | Glucosamine | — | — | — | — | — | — | (6) | — | — | — | — | — | — | — | — | 5 | 3 | (2) | 3 |
| 17.23 | Inositol | — | — | — | — | — | — | (3) | — | — | — | — | — | — | — | — | (2) | — | 5 | — |
| 17.76 | $C_5$-phenyl | — | — | — | — | (3) | — | — | — | — | — | — | — | — | (3) | — | (3) | 3 | 3 | 4 |
| 18.11 | Galactosamine | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 4 | 4 | 4 | 3 |
| 18.46 | Galactosamine | — | — | — | — | — | — | (4) | — | — | — | — | — | — | — | — | 3 | 2 | (3) | (2) |
| 18.54 | Glucosamine | — | — | — | — | — | — | — | — | (3) | — | — | — | — | — | — | (5) | — | (5) | (3) |
| 18.59 | Mannosamine | — | — | — | — | — | — | (7) | — | — | — | — | — | — | — | — | (5) | 4 | (5) | 3 |
| 18.75 | Galactosamine (?) | (3) | (3) | (3) | — | 4 | — | — | — | 3 | 3 | — | — | — | — | 4 | 4 | 5 | 4 | (4) |

[a] Abbreviations for reaction types: A, peracetylated aldononitrile; O, peracetylated O-methyloxime; $C_5$ and $C_6$, unknown carbohydrates with five and six carbon atoms, respectively; -ol, sugar alcohol; phenyl, phenylated carbohydrate; $CH_3$, methylated carbohydrate. When no reaction type is specified, only acetylation was observed (with sugar alcohols, amino sugars, and similar compounds). ?, identity of carbohydrate was uncertain.

[b] 1, 0.1 to 0.5%; 2, 0.5 to 1.0%; 3, 1.1 to 3.0%; 4, 3.1 to 6.0%; 5, 6.1 to 10.0%; 6, 10.1 to 20.0%; 7, 20.1 to 30.0%; 8, more than 30.0%; —, not detected. Values in parentheses indicate that the reactions were variable (carbohydrate was not detected in all samples); at least five samples were examined for each carbohydrate.

[c] Preliminary data have been reported previously (36).

*O. rhinotracheale* can be further differentiated from *R. anatipestifer* by the absence of catalase activity and by the presence of β-glucosaminidase activity and α- and β-galactosidase activity (*R. anatipestifer* strains have the opposite reactions for these tests [42]). Additional characteristics were determined by using experimental API galleries (Table 7). *O. rhinotracheale* strains do not exhibit gamma-glutamyl arylamidase activity, and most strains produce acid from fructose (18 of 21 strains), lactose (19 of 21 strains), galactose (15 of 16 strains), and *N*-acetyl-glucosamine (15 of 16 strains), whereas the five reference strains of *R. anatipestifer* have the opposite reactions. The absence of catalase activity and the presence of oxidase activity should allow workers to differentiate *O. rhinotracheale* from *Capnocytophaga* species; *Capnocytophaga sputigena*, *Capnocytophaga gingivalis*, and *Capnocytophaga ochracea* do not exhibit catalase or oxidase activity, while *Capnocytophaga canimorsus* and *Capnocytophaga cynodegmi* produce both catalase and oxidase (4). Additional characteristics are shown in Table 7.

A relatively new approach in bacterial taxonomy is the determination of cellular carbohydrate contents (3, 37). The presence of an unidentified $C_5$ compound with retention times of 11.44 and 11.98 min, the presence of a $C_6$ compound with a retention time of 15.28 min, the presence of ribose, the presence of large amounts of lyxose, mannose, glucose, and galactose, and the lack of arabinose (except in strains LMG 10969, LMG 12589, LMG 12591, and LMG 12599), sorbose, and heptoses are general properties of organisms belonging to rRNA superfamily V, whereas this general carbohydrate pattern has not been observed in members of the other rRNA superfamilies investigated so far (21).

3-Deoxy-D-mannooctulosonic acid was not observed. This could have been because of the derivatization technique used, which was not optimal for this compound, or because there was a proportionally low level of 3-deoxy-D-mannooctulosonic acid present. The differences determined for the hexosamines glucosamine, galactosamine, and mannosamine are of minor taxonomic importance.

**Description of *Ornithobacterium* gen. nov.** *Ornithobacterium* (Or.ni.tho.bac.te'ri.um. Gr. n. *ornis*, bird; Gr. neut. n. *bakterion*, rod; N. L. neut. n. *Ornithobacterium*, bird bacterium, because the organism was first isolated from birds). *Ornithobacterium* cells are gram-negative, nonmotile, nonsporulating, short, plump rods with chemoorganotrophic, mesophilic metabolism. *Ornithobacterium* strains grow under various atmospheric conditions at temperatures between 30 and 42°C. Colonies are not pigmented on common growth media. Menaquinone 7 is the sole respiratory quinone. The branched fatty acids 15:0 iso, 15:0 iso 3OH, and 17:0 iso 3OH are the major fatty acid components. The DNA base composition ranges from 37 to 39 mol% G+C. The type species is *O. rhinotracheale* sp. nov.

**Description of *Ornithobacterium rhinotracheale* sp. nov.** *Ornithobacterium rhinotracheale* (rhi.no.tra.che.a'le. Gr. n. *ris* nose, nostril; medical term *trachea*, windpipe; L. neut. adj. suff. *-ale*, pertaining to; N. L. neut. adj. *rhinotracheale*, relating to nostrils and windpipes, because the organism was first isolated from specimens obtained from windpipes and nostrils). *O. rhinotracheale* cells are 0.2 to 0.9 μm wide and 1 to 3 μm long. Most strains grow aerobically, microaerobically, anaerobically, and in a $CO_2$-enriched atmosphere. Growth occurs at 30, 35, and 42°C. Weak or no growth occurs at 24°C. After storage for 6 weeks at 6 to 8°C, blood agar cultures can be subcultured on blood agar. Colonial adherence, spreading, and corrosion do not occur. No hemolysis occurs on horse blood agar. Smooth, nonpigmented colonies develop after 2 days of incubation on rich peptone, peptone-blood, or chocolate agar at 36°C. Oxidase activity is present in most strains; catalase activity is absent. No growth occurs on MacConkey agar, Endo agar, Drigalski agar, or Simmons citrate medium. No growth factors are required. Alkaline phosphatase and β-galactosidase (*o*-nitrophenyl-β-D-galactopyranoside test) are present. Nitrates are not reduced; some strains reduce nitrites, but no denitrification occurs. Arginine dihydrolase is present in most strains if the strains are grown in Möller medium (Difco) after 3 days of incubation at 36°C. Lysine and ornithine decarboxylase, phenylalanine deaminase, lecithinase, DNase, and gelatinase activities are absent. Urease activity as determined by Lautrop's nonproliferative test (30) is present. Indole is not produced. Hydrogen sulfide is not detected in Kligler's agar, triple sugar iron agar, or SIM agar. Esculin is not hydrolyzed. Acetylmethylcarbinol is produced in the Voges-Proskauer test. Methyl red test negative. Hyaluronidase and chondroitin sulfatase activities are present. Ethanol is not oxidized. D-Glucose in oxidation-fermentation medium is weakly and slowly oxidized or not detected. Wheat starch is acidified by most strains. Most strains use D-galactose, D-glucose, D-mannose, lactose, and sucrose as carbon sources. D-Xylose, D-mannitol, D-sorbitol, and malonate are not used by most strains as carbon sources. In general, carbohydrates are catabolized better in media supplemented with 2% chicken serum. Wheat starch is acidified by most strains within 1 to 5 days if cultures are incubated at 36°C.

The following enzyme activities are always present: alkaline and acid phosphatase, ester lipase C8, leucine arylamidase, phosphoamidase, α-glucosidase, β-glucosaminidase, phosphodiesterase, alanine arylamidase, glycine arylamidase, lysine arylamidase, proline arylamidase, α-glutamyl-α-glutamic acid arylamidase, glycyl-phenylalanine arylamidase, phenylalanyl-arginine arylamidase, prolyl-arginine arylamidase, seryl-methionine arylamidase, 2-glycyl-glycyl-arginine arylamidase, and alanyl-phenylalanyl-prolyl-alanine arylamidase. All strains exhibit strong or weak α- and β-galactosidase, esterase C4, valine and cysteine arylamidase, and trypsin activities. The following enzyme activities are always absent: β-glucuronidase, β-glucosidase, α-mannosidase, α-fucosidase, lipase C14, phenylalanine deaminase, hippurate hydrolysis, gamma-glutamyl arylamidase, and phospholipase. No acid is produced from D-glucosaminic acid, D-saccharic acid, L-fucose, D-mannitol, D-sorbitol, trehalose, and D-xylose.

The additional fatty acids present in small quantities in all strains include two unidentified fatty acids with equivalent chain lengths of 13.566 and 16.580, 16:0, 17:0 iso, and 16:0 3OH.

Lyxose, ribose, glucose, galactose, and mannose are the principal carbohydrate components, while altrose, sorbose, and heptose do not occur.

Strains have been isolated from respiratory tracts of turkeys, chickens, rooks, and a partridge, and most strains have been associated with infections such as tracheitis, pericarditis, sinusitis, airsacculitis, and pneumonia. At present, our knowledge with regard to pathogenicity in birds is incomplete. Our clinical observations and the results of diagnostic work (unpublished data) indicate with a high level of probability that *O. rhinotracheale* is able to produce a contagious disease. However, it is not yet clear whether cofactors are involved in pathogenesis.

The DNA base composition ranges from 37 to 39 mol% G+C. The type strain is LMG 9086 (= MCCM 01774 = CCUG 23171), which was isolated from a turkey in the United Kingdom. Its G+C content is 38 mol%.

All *O. rhinotracheale* strains have been deposited in the Culture Collection of the Laboratorium voor Microbiologie and the Culture Collection of the University of Göteborg Department of Clinical Bacteriology.

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