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Bordetella avium sp. nov., Isolated from the Respiratory Tracts of Turkeys and Other Birds

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Bordetella avium is proposed as the name of a new species containing 28 avian strains that cause coryza (rhinotracheitis) in turkey poults. The type strain is Hinz 591-77 (= ATCC 35086). The majority of the strains investigated were previously known as *Bordetella*-like or *Bordetella bronchiseptica*-like bacteria; one of the strains was previously referred to as *Alcaligenes faecalis*. The *B. avium* strains were compared with more than 50 culture collection strains belonging to *Bordetella bronchiseptica, Bordetella pertussis, Bordetella parapertussis, Alcaligenes faecalis, "Alcaligenes odorans," Alcaligenes denitrificans, Achromobacter xylosoxidans, Pseudomonas pertucinogena*, and unnamed groups IVc-2 and IVe. The properties of *B. avium* and its taxonomic position relative to the above-mentioned taxa were determined by morphological, physiological, nutritional, and serological studies and by a numerical analysis of protein electropherograms, deoxyribonucleic acid-ribosomal ribonucleic acid hybridizations, and pathogenicity tests for turkey poults. The 28 *B. avium* strains formed a tight cluster, sharing very similar phenotypic features and protein gel electropherograms. We observed no significant differences among strains isolated from turkeys in different geographical areas. The 28 strains were strictly aerobic, gram-negative, peritrichously flagellated, urease-negative rods; the deoxyribonucleic acid base composition ranged from 61.6 to 62.6 mol% guanine plus cytosine. The phenotypic and serological characteristics, together with the properties of the deoxyribonucleic acid-ribosomal ribonucleic acid hybrids, indicated that this new species is a member of the genus *Bordetella*. The *Alcaligenes denitrificans-Achromobacter xylosoxidans* cluster is the closest neighbor of *Bordetella*, which is clearly different from all of the other taxa examined. *B. avium* has been isolated from the respiratory tracts of turkeys and from some other birds, such as a chicken, a duck, and a goose. All of the *B. avium* strains investigated caused coryza in turkey poults. An extensive phenotypic description of *B. avium* is given, and this species is differentiated phenotypically from the following taxa: *B. pertussis, B. parapertussis, B. bronchiseptica, Alcaligenes faecalis, Alcaligenes denitrificans, Achromobacter xylosoxidans*, and groups IVc-2 and IVe.

Filion et al. (15) described a respiratory disease in turkey poults in Canada that was caused by glucose-nonfermenting bacteria. The same disease was later reported in the United States, the Federal Republic of Germany, and other countries and was variously named turkey coryza (26, 53), rhinotracheitis (56, 57), and bordetellosis (24). On the basis of morphological and biochemical features, Simmons et al. (58) identified the agent of rhinotracheitis in turkeys as *Alcaligenes faecalis*, whereas Hinz et al. (23) considered these bacteria to be related to the genus *Bordetella* and designated them temporarily *Bordetella bronchiseptica*-like or *Bordetella*-like bacteria (K.-H. Hinz, Abstr. 7th Int. Congr. World Vet. Poultry Assoc., p. 79, 1981).

The aim of the present study was to examine the taxonomic relationships of 28 *Bordetella*-like strains which were isolated from birds and caused coryza (rhinotracheitis) in turkey poults. The morphological, physiological, and biochemical features of these strains were investigated, and the deoxyribonucleic acid (DNA) base compositions were determined. The strains were also compared by polyacrylamide gel electrophoresis of their proteins, pathogenicity tests, and agglutination and gel diffusion precipitation tests. Hybridizations between DNA and ribosomal ribonucleic acid (rRNA) were performed to determine the relationships between selected *Bordetella*-like strains and representative strains belonging to the following taxa for which there is evidence of possible affinity (quotation marks indicate that the name is not on the Approved Lists of Bacterial Names [60] or on the Validation Lists): *Alcaligenes faecalis, "Alcaligenes odorans," Alcaligenes denitrificans, Achromobacter xylosoxidans, B. bronchiseptica, Bordetella parapertussis, Bordetella pertussis*, and *Alcaligenes*-like and *B. bronchiseptica*-like strains assigned at the Centers for Disease Control (CDC), Atlanta, Ga., to groups IVc-2 and IVe. We present evidence that the turkey coryza agent constitutes a new species, which we name *Bordetella avium*.

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MATERIALS AND METHODS

Bacterial strains. The designations and sources of the strains used are listed in Table 1. All of the strains were checked for purity by plating and microscopic examination of Gram-stained cells. The *B. avium* strains were stored in a freeze-dried state until they were used. For the majority of the experiments, working cultures were maintained at −70°C as suspensions in steamed milk. Before a test series was begun, the frozen cultures were thawed, and one passage was made on blood agar containing Columbia agar base (Oxoid Ltd., London, United Kingdom) and 7% defibrinated ox blood or on veal infusion (VI) agar (Difco Laboratories, Detroit, Mich.). The strains used for DNA extraction, auxanography, and electrophoresis of native soluble proteins were maintained on nutrient agar slants at 4°C.

Morphological and growth characteristics. The colony morphology of the *B. avium* strains was examined on blood agar and VI agar after 24 h of incubation at 35°C in moist air. Flagellar staining (64) was carried out with bacterial cells that were harvested from blood agar cultures after 24 h of

incubation at room temperature. Inoculated blood agar and VI agar plates were incubated aerobically and anaerobically (GasPak 110 System; BBL Microbiology Systems, Cockeysville, Md.) for 2 and 5 days. Salmonella-shigella agar (BBL) and MacConkey agar (E. Merck AG, Darmstadt, Federal Republic of Germany) were prepared according to the directions of the manufacturers. Growth was recorded after 5 days of incubation at 35°C.

Biochemical and physiological tests. Oxidase activity was tested on 18- to 24-h cultures with freshly prepared Kovács (34) reagent and with a less sensitive solution containing 0.2 ml of 1% α-naphthol in 95% ethanol and 0.3 ml of 1% *N,N*-dimethyl-1,4-phenylenediammonium dichloride (Merck). Two or three drops of the reagent were placed on a piece of filter paper in a petri dish, and some colonies were removed with a cover slip and smeared across the surface of the impregnated paper. The reactions were read within 30 s when the Kovács reagent was used and within 2 min when the *N,N*-dimethyl-1,4-phenylenediammonium dichloride reagent was used. Catalase activity was determined by using VI agar cultures; a few colonies were removed with a cover slip and then mixed with 1 drop of 3% H_2O_2. The evolution of gas bubbles within 2 min was recorded as a positive test.

Most of the other biochemical tests were performed according to standard methods (4). Unless otherwise indicated, the inoculated test media were incubated at 35°C in an aerobic environment for 5 days and were read daily. Urea hydrolysis was observed on Christensen urea agar (Merck). Nitrate reduction was determined on 5 consecutive days of incubation in nitrate broth (Merck). Negative reactions were confirmed by adding zinc dust. The ability to utilize citrate as a carbon source was tested on Simmons citrate agar (Oxoid) and Christensen citrate agar (Merck). For the methyl red test, methyl red-Voges-Proskauer broth (Merck) was used, and cultures were tested after 1 and 5 days of incubation by adding methyl red. Indole production was tested in Standard II nutrient broth (Merck) after 2 and 5 days by adding 1 ml of Kovács indole reagent (Merck) to 5 ml of a broth culture. Gelatin hydrolysis was tested by flooding plates with Frazier (16) reagent. Esculin hydrolysis was determined in esculin broth (4). Phenylalanine deamination was determined after 16 to 20 h of incubation in malonate phenylalanine broth (Merck) (55) by adding 0.2 ml of a 10% $FeCl_3$ solution to a broth culture acidified with 0.1 to 0.2 ml of 1 N HCl. Alkalinization of litmus milk was tested in steamed milk containing 7% Kubel-Tiemann litmus solution (Merck). To examine the oxidative-fermentative utilization of glucose, we used CDC oxidation-fermentation medium at pH 7.3 (46) supplemented with bromothymol blue as the indicator (2.5 ml of a 0.4% aqueous bromothymol blue solution in 100 ml of medium). Duplicate tubes were inoculated by stabbing with a straight wire. To one of the tubes a layer of molten soft paraffin was added to a depth of 1 cm. Other enzymes were investigated by using the API-ZYM System (API Labor System GmbH, Wiesbaden, Federal Republic of Germany). Overnight cultures on VI agar were washed twice and then suspended in sterile distilled water to no. 5 turbidity on the McFarland scale. After 4 h of incubation at 37°C, test reagents were added according to the instructions of the manufacturer. Alkalinization of amides and organic salts was tested in Greenwood low-peptone medium (pH 6.5) containing bromothymol blue instead of phenol red as the indicator (46). Susceptibility to penicillin G was tested by using the disk method on diagnostic sensitivity agar (Oxoid). The plates were incubated at 35°C for 16 to 18 h and were examined for zone inhibition.

Carbon substrate utilization tests. The utilization of 147 organic compounds as sole carbon sources was investigated by using the following three API galleries: API 50CH (carbohydrates), API 50AO (organic acids), and API 50AA (amino acids and amines) (API System, La Balme-les-Grottes, Montalieu-Vercieu, France). This auxanographic technique has been used by Gavini et al. (17) for the *Enterobacteriaceae*. A standardized procedure was used to ensure that the tests were comparable between strains. Each strain was grown for 48 h on a nutrient agar (Oxoid) slant at 30°C. However, *B. parapertussis* strains had to be grown on brain heart infusion (Oxoid) slants. The medium for the auxanographic tests was prepared by autoclaving 55-ml portions of 0.1 M phosphate buffer (pH 7.0) containing 0.5% Oxoid no. 1 agar in 100-ml flasks. The medium was maintained at 42°C in a water bath in order to keep it in a liquid form, and 5 ml of a filter-sterilized solution containing 8% yeast nitrogen base (Difco) and 0.6% yeast extract (Oxoid) in distilled water was added aseptically. A loopful of bacteria was taken from the slant and suspended in 3 ml of distilled water. The turbidity of the suspension was adjusted to 5 to 6 on the McFarland scale. The inoculum was thoroughly mixed with the medium, and each cupule of the test galleries was inoculated by using a sterile Pasteur pipette. Readings were made after 1, 2, 4, and 7 days of incubation at 30°C. Reactions were recorded as positive whenever visible growth occurred. There was no growth in the control cupule without a carbon source.

Polyacrylamide gel electrophoresis of soluble proteins. Bacteria (about 2 g, wet weight) were grown in Roux flasks at 28°C for 40 h on medium containing 10 g of peptone (Oxoid) per liter, 8 g of Lab-Lemco powder (Oxoid) per liter, 5 g of NaCl per liter, and 25 g of agar per liter in tap water. The preparation of cell-free extracts, polyacrylamide gel electrophoresis of the soluble proteins, densitometry, normalization of the densitometric tracings, and photography of the stained gels were carried out as described by Kersters and De Ley (30). Each protein extract was investigated in at least three independent electrophoretic runs. The normalized densitometric tracings were converted into a sequence of 120 numbers, which represented the optical density (expressed in millimeters) of each position on a scan (30). The Pearson product-moment correlation coefficient (r) between each pair of densitometric tracings was calculated, and the protein electropherograms were grouped by the unweighted average pair group method, using the Clustan program (version 1C) of Wishart (65) and a Siemens model 7541 (BS2000) computer at the Centraal Digitaal Rekencentrum, Rijksuniversiteit, Ghent, Belgium. The most typical electropherogram of each strain was calculated as described previously (63). In the final numerical analysis only the most representative electropherogram for each strain was used.

The soluble proteins obtained by sonicating bacterial suspensions were also compared by polyacrylamide flat gel electrophoresis, using sodium dodecyl sulfate-containing gradient gels with 7.5 to 15% acrylamide (35). The apparatus used was that of Studier (62), as modified by Neumann and Hinz (43). About 20 μg of bacterial protein per sample gave optimal resolution. Electrophoresis was carried out at room temperature for 5 h at a constant current of 18 mA; the voltage was 50 V at the beginning and 130 V at the end of the run. The gel slabs were stained with Coomassie blue R250 (Serva, Heidelberg, Federal Republic of Germany).

Preparation of labeled rRNA. 23S [^{3}H]rRNA was prepared as described previously (7, 11) from *B. bronchiseptica* NCTC 452^T (= ATCC 19395^T) (T = type strain), and 23S [^{14}C]rRNAs were prepared from *Alcaligenes faecalis* NCIB

TABLE 1. Strains used

Name as received	Strain no.[a]	Received from[a]:	Source[b]
Strains assigned by us to *Bordetella avium*			
Bordetella-like strain	591-77^T	Our isolate	Air sac exudate of turkey, FRG
Bordetella-like strain	946-77	Our isolate	Lung of turkey, FRG
Bordetella-like strain	110-78	Our isolate	Lung of turkey, FRG
Bordetella-like strain	146-78	Our isolate	Infraorbital sinus of chicken, FRG
Bordetella-like strain	363-78	Our isolate	Trachea of turkey, FRG
Bordetella-like strain	373-78	Our isolate	Trachea of turkey, FRG
Bordetella-like strain	383-78	Our isolate	Trachea of turkey, FRG
Bordetella-like strain	450-78	Our isolate	Liver of sharp-tailed munia, FRG
Bordetella-like strain	615-78	Our isolate	Lung of turkey, FRG
Bordetella-like strain	700-78	Our isolate	Lung of turkey, FRG
Bordetella-like strain	731-78	Our isolate	Trachea of turkey, FRG
Bordetella-like strain	270-80	Our isolate	Lung of goose, FRG
Bordetella-like strain	2334-80	Our isolate	Trachea of turkey, FRG
Bordetella-like strain	2-81	Wilding	Turkey, UK
Bordetella-like strain	3-81	Wilding	Turkey, UK
Bordetella-like strain	17-81	Wilding	Turkey, UK
Bordetella-like strain	18-81	Wilding	Turkey, UK
Bordetella-like strain	21-81	Wilding	Turkey, UK
Bordetella-like strain	114-81	Our isolate	Trachea of turkey, FRG
Bordetella-like strain	298-81	Our isolate	Trachea of duck, FRG
Bordetella-like strain	XX14	Dolz	Turkey, Spain
Bordetella-like strain	XX15	Dolz	Turkey, Spain
Bordetella-like strain	676	Bendheim	Turkey, Israel
Bordetella-like strain	897	Bendheim	Turkey, Israel
Bordetella-like strain	1106	Bendheim	Turkey, Israel
Bordetella-like strain	2646	Bendheim	Turkey, Israel
Bordetella-like strain	P4085	Rimler	Turkey, United States
(*Alcaligenes faecalis*)[c]	NC	Simmons	Turkey, United States
Reference strains			
Bordetella bronchiseptica	NCTC 452^T (= ATCC 19395^T)	NCTC	Lung of dog
B. bronchiseptica	NCTC 454	NCTC	Blood of rabbit
B. bronchiseptica	NCTC 455	NCTC	Trachea of ferret
B. bronchiseptica	NCTC 456	NCTC	Trachea of monkey
B. bronchiseptica	NCTC 458	NCTC	Sputum of human
B. bronchiseptica	NCTC 1704	NCTC	Blood culture
B. bronchiseptica	CCM 6047	CCM	Pig
B. bronchiseptica	NCTC 8344	NCTC	
B. bronchiseptica	NCTC 8761	NCTC	Upper respiratory tract
B. bronchiseptica	NCTC 8762	NCTC	Upper respiratory tract
B. bronchiseptica	NCTC 10539	NCTC	Whooping cough-like disease, child
B. bronchiseptica	NCTC 10541	NCTC	Rabbit
B. bronchiseptica	NCTC 10542	NCTC	Nasal discharge of rabbit
B. bronchiseptica	40-81	Our isolate	Trachea of turkey, FRG
B. parapertussis	NCTC 5952^T	NCTC	Whooping cough
B. parapertussis	NCTC 7385	NCTC	
B. pertussis	NCTC 10739^T (= ATCC 9797^T)[d]	Mannheim	
B. pertussis	NCTC 8189[d]	Mannheim	Whooping cough
Achromobacter xylosoxidans	KM 543^T (= ATCC 27061^T)	Yabuuchi	Human ear discharge, Japan
A. xylosoxidans	KM 563 (= ATCC 27062)	Yabuuchi	Human ear discharge, Japan
A. xylosoxidans	KM 790	Yabuuchi	Human ear discharge, Japan
A. xylosoxidans	KM 949 (= CDC B4043)	Yabuuchi	Human spinal fluid
A. xylosoxidans	KM 950 (= CDC B5140)	Yabuuchi	Human sputum
A. xylosoxidans	KM 954 (= CDC B6949)	Yabuuchi	Human skin
A. xylosoxidans	KM 956 (= CDC B7076)	Yabuuchi	Human stool
Achromobacter sp.	M250 (= ATCC 9220)	Pickett	
Alcaligenes denitrificans	CIP 58.72 (= CIP X73)	CIP	Human sputum, France
A. denitrificans	CIP 61.20 (= CIP X86)	CIP	Disinfectant, France
A. denitrificans	CIP 60.83	CIP	Human urine
A. denitrificans[e]	ATCC 15173^T (= NCTC 8582^T)[e]	ATCC and NCTC	Soil
A. faecalis	R161	CIP	Human blood culture, France
A. faecalis	CCM 267	CCM	Salted leather
A. faecalis	NCTC 415 (= ATCC 19018)	NCTC	Human feces
A. faecalis	P4083	Rimler	Turkey, United States
A. faecalis	NCIB 8156^T (= ATCC 8750^T = CCM 1052^T)	NCIB and CCM	
A. faecalis	AB 78 (= NCTC 8764)	Lautrop	Human blood culture
(*A. faecalis*)	CIP 57.58	CIP	Human sputum

TABLE 1—*Continued*

Name as received	Strain no.[a]	Received from[a]:	Source[b]
"*A. odorans* var. *viridans*"	Mit 1 (= ATCC 19209 = NCTC 10388)[f]	Mitchell	Swab from human wound
"*A. odorans* var. *viridans*"	Mit 5	Mitchell	
"*A. odorans*"	AB54	Lautrop	
"*A. odorans* var. *viridans*"	Gilardi 79	Gilardi	Human urine
"*A. odorans* var. *viridans*"	Gilardi 117	Gilardi	
"*A. odorans*"	CCEB 554 (= ATCC 15554)[g]	CCEB	Feces
"*A. odorans*"	CCEB 568	CCEB	Nematode
"*A. odorans*"	CCEB 569	CCEB	Nematode
Alcaligenes sp.	AB1374	Lautrop	Swab from toe wound
Pseudomonas pertucinogena	KM 1319^T (= ATCC 190^T)	Yabuuchi	Probably from human respiratory tract
Group IVc-2	CDC E6793	Weaver	Human respiratory tract, Oregon
Group IVc-2	CDC E8967	Weaver	Human sputum, Georgia
Group IVe	CDC F1147	Weaver	Human urine, Alaska
Group IVe	CDC B8375	Weaver	Human urine, California

[a] ATCC, American Type Culture Collection, Rockville, Md.; CCEB, Culture Collection of Entomogenous Bacteria, Prague, Czechoslovakia; CCM, Czechoslovak Collection of Microorganisms, Brno, Czechoslovakia; CDC, Centers for Disease Control, Atlanta, Ga.; CIP, Collection de l'Institut Pasteur, Paris, France; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, United Kingdom; Bendheim, U. Bendheim, Institute of Poultry Diseases, Jerusalem, Israel; Dolz, M. A. Dolz, División Aveselectas, Tortosa, Spain; Gilardi, G. L. Gilardi, Hospital for Joint Diseases and Medical Center, New York, N.Y.; Lautrop, H. Lautrop, Statens Serum Institutet, Copenhagen, Denmark; Mitchell, R. Mitchell, The United Oxford Hospitals, Oxford, United Kingdom; Pickett, M. J. Pickett, Department of Microbiology, University of California, Los Angeles; Rimler, R. B. Rimler, National Animal Disease Center, Ames, Iowa; Simmons, D. G. Simmons, School of Veterinary Medicine, North Carolina State University, Raleigh; Weaver, R. E. Weaver, General Bacteriology Branch, Centers for Disease Control, Atlanta, Ga.; Wilding, G. P. Wilding, British United Turkeys Ltd., Veterinary Laboratory, Chester, United Kingdom; Yabuuchi, E. Yabuuchi, Department of Microbiology, Kansai Medical University, Osaka, Japan; Mannheim, W. Mannheim, Klinikum der Philipps-Universität Marburg, Marburg, Federal Republic of Germany; NCIB, National Collection of Industrial Bacteria, Torrey Research Station, Aberdeen, United Kingdom.

[b] FRG, Federal Republic of Germany; UK, United Kingdom.

[c] Brackets indicate that the strain has been misnamed.

[d] *B. pertussis* strains were cultivated by W. Mannheim.

[e] Recently revived by Rüger and Tan (52) with strain ATCC 15173 as the type strain, which was also previously designated by Leifson and Hugh (36) and Sneath and Skerman (61) as the type strain of *Alcaligenes denitrificans*.

[f] Previously designated as the type strain of "*Alcaligenes odorans* subsp. *viridans*" (41).

[g] Previously designated the type strain of "*Alcaligenes odorans*" (38).

8156^T (= ATCC 8750^T) and *Alcaligenes denitrificans* ATCC 15173^T. To prepare the labeled rRNA, the two *Alcaligenes* strains were grown on the medium described by De Ley et al. (8); *B. bronchiseptica* NCTC 452^T was grown on the same medium containing 2 mCi of [^{3}H]uracil instead of 100 μCi of [^{14}C]uracil.

Preparation of DNA, saturation hybridization between labeled rRNA and filter-fixed DNA, and determination of the thermal stability of the DNA-rRNA hybrids. We used the methods described previously (7, 11, 19).

Determination of DNA base composition (G+C content). The average guanine-plus-cytosine (G + C) content of each DNA was measured by the thermal denaturation method (9) and was calculated by the equation of Marmur and Doty (39) as modified by De Ley (5).

Pathogenicity tests. The 28 avian strains, 2 *B. bronchiseptica* strains and 2 *Alcaligenes faecalis* strains were tested for pathogenicity on turkey poults. For these tests 1-day-old turkey poults were obtained from a commercial parent breeder flock; these birds were free of *Mycoplasma gallisepticum* and *Mycoplasma synoviae*. No agglutinating antibodies against the thermolabile antigen of the bacterial turkey coryza agent were detected in the poults from this flock.

Five poults (3 days old) were inoculated intranasally with about 10^4 colony-forming units of one of the strains. Immediately after inoculation the five birds were placed in direct contact with 5 to 10 non-inoculated poults. Each of the groups was kept under optimal conditions in a separate negative-pressure isolation unit (size, 80 by 150 cm). The poults were examined for clinical signs for 10 to 12 days. The examinations included observation and gentle manual compression of the upper beak in the region of nasal turbinates. After the poults were killed, they were examined for macroscopic lesions, and the tracheas were checked for the presence of bacteria by scraping the mucosas of the tracheas with an inoculating loop and making streaks on blood agar.

Serological tests. Antisera were prepared by immunizing rabbits against eight *B. avium* strains as described by Hinz (22). Agglutination tests and agglutinin absorption tests were carried out as described by Hinz et al. (25) and Pedersen (44). Sonicated whole cell antigen was used for the gel diffusion test. The growth from VI agar plates was suspended in a 0.01 M phosphate-buffered saline solution (pH 7.2) and adjusted to a cell concentration of 15% (vol/vol). The bacterial suspensions were ruptured by sonication for 3 min and then centrifuged at 10,000 × *g* for 20 min. The supernatant was removed and used as an antigen. Gel diffusion precipitation tests were carried out as described by Hinz (22).

RESULTS

Morphological, physiological, and biochemical features of *B. avium*. The 28 strains assigned to *B. avium* (Table 1) were strictly aerobic, gram-negative, capsulated rods with an

TABLE 2. Features present in *B. avium*

Test	Positive results obtained for:
Alkalinization[a]	Acetamide, asparagine, formamide, glutamine, propionamide, succinamide, acetate, adipate, citrate, formate, propionate
Production of enzymes[b,c]	Acid phosphatase, alkaline phosphatase (weak), esterase (C-4), esterase-lipase (C-8) (weak), L-leucyl-2-naphthylamide hydrolase, phosphoamidase (weak)
Growth on carbon sources[b,d]	
Dicarboxylic acids	Fumarate, glutarate, succinate
Hydroxy acids	DL-3-Hydroxybutyrate (weak), DL-lactate, L-malate
Miscellaneous organic acids	Citrate, α-ketoglutarate, pyruvate
Amino acids	L-Aspartate, L-glutamate, L-proline

[a] We investigated 28 strains.
[b] We investigated eight strains, including type strain 591-77.
[c] Tested with API ZYM strips.
[d] Positive reactions were obtained with API 50CH, API 50AO, and API 50AA galleries after 7 days of incubation at 30°C. Some strains grew poorly on fumarate, glutarate, L-malate, citrate, α-ketoglutarate, L-aspartate, L-glutamate, and L-proline.

average diameter of 0.4 to 0.5 μm and an average length of 1 to 2 μm; these cells were arranged singly or in pairs. Growth occurred on MacConkey agar, salmonella-shigella agar, and nutrient agar. When the *B. avium* strains were grown on blood agar and VI agar, two distinct colony types were observed. Type I colonies (16 strains) were small, compact, and pearllike with entire edges and glistening surfaces and were less than 1 mm in diameter after 24 h of incubation. Type II colonies were larger, circular, and convex with entire edges and smooth surfaces. Type I colonies often had brownish centers on VI agar. Only three *B. avium* strains showed dissociation, whereas all of the other strains remained stable even after several transfers.

All *B. avium* strains were motile, with five to eight peritrichous flagella per cell. The number of motile rods was significantly greater in cultures that were incubated at room temperature than in cultures that were incubated at 35°C. This phenomenon was not observed for the *Alcaligenes faecalis* and *B. bronchiseptica* strains examined.

No pigments were produced. The strains grew optimally at 37°C. All of the strains yielded positive reactions in the catalase, Kovács oxidase (34), and Simmons citrate tests. They did not produce indole, urease, or phenylalanine deaminase. None of the strains hydrolyzed esculin or gelatin. Nitrate was not reduced, and the strains did not produce acid or alkali in glucose-containing CDC oxidation-fermentation medium. Other biochemical and nutritional features are summarized in Tables 2 to 4. Since the *B. avium* strains constituted a homogeneous group of organisms as determined by protein electrophoresis (see below), some tests were performed on eight representative strains, including the proposed type strain, strain 591-77. A total of 190 features were common to all of the *B. avium* strains investigated

TABLE 3. Features absent in *B. avium*

Test	Negative results obtained for:
Alkalinization[a]	Malonamide, valeramide, maleate, malonate, mucate, saccharate, valerate, litmus milk
Production of enzymes[b,c]	*N*-Acetyl-β-D-glucosaminidase, *N*-benzoyl-DL-arginine-2-naphthylamide hydrolase (trypsin), L-cystyl-2-naphthylamide hydrolase, α-L-fucosidase, α-D-galactosidase, β-D-galactosidase, α-D-glucosidase, β-D-glucosidase, β-D-glucuronidase, lipase (C14), α-D-mannosidase, L-valyl-2-naphthylamide hydrolase
Growth on carbon sources[b,d]	
Carbohydrates and sugar acids	*N*-Acetyl-glucosamine, amygdalin, D-arabinose, L-arabinose, arbutin, D-cellobiose, esculin, D-fructose, D-fucose, L-fucose, D-galactose, D-gentiobiose, D-gluconate, D-glucosamine, D-glucose, glycogen, inulin, 2-keto-gluconate, 5-keto-gluconate, lactose, D-lyxose, D-maltose, D-mannose, D-melezitose, D-melibiose, methyl-α-D-glucoside, methyl-α-D-mannoside, methyl-β-D-xyloside, raffinose, L-rhamnose, D-ribose, salicin, L-sorbose, starch, sucrose, D-tagatose, trehalose, D-turanose, D-xylose, L-xylose
Polyols	Adonitol, D-arabitol, L-arabitol, dulcitol, *meso*-erythritol, glycerol, *meso*-inositol, D-mannitol, sorbitol, *meso*-xylitol
Fatty acids	Butyrate, caprate, *n*-caproate, caprylate, heptanoate, isobutyrate, isovalerate, pelargonate, propionate, *n*-valerate
Dicarboxylic acids	Azelate, maleate, malonate, oxalate, pimelate, sebacate, suberate
Hydroxy acids	DL-Glycerate, glycolate, D-malate, D-tartrate, L-tartrate, *meso*-tartrate
Miscellaneous organic acids	Aconitate, citraconate, itaconate, levulinate, mesaconate
Nonnitrogenous aromatic and other cyclic compounds	Benzoate, *o*-hydroxybenzoate, *m*-hydroxybenzoate, *p*-hydroxybenzoate, D-mandelate, L-mandelate, phthalate, *iso*-phthalate, *tere*-phthalate
Amino acids and related compounds	D-α-Alanine, L-α-alanine, β-alanine, L-arginine, L-citrulline, L-cysteine, glycine, L-histidine, L-isoleucine, DL-kynurenine, L-leucine, L-lysine, L-methionine, L-norleucine, DL-norvaline, L-ornithine, L-phenylalanine, L-serine, L-threonine, trigonelline, D-tryptophan, L-tryptophan, L-valine
Amines and miscellaneous nitrogenous compounds	Acetamide, 2-aminobenzoate, 3-aminobenzoate, 4-aminobenzoate, DL-2-aminobutyrate, DL-3-aminobutyrate, DL-4-aminobutyrate, DL-5-aminovalerate, amylamine, benzylamine, betaine, butylamine, creatine, diaminobutane, ethanolamine, ethylamine, histamine, sarcosine, spermine, tryptamine, urea

[a] We investigated 28 strains.
[b] We investigated eight strains, including type strain 591-77.
[c] Tested with API ZYM strips.
[d] Negative reactions were obtained with API 50CH, API 50AO, and API 50AA galleries after 7 days of incubation at 30°C.

TABLE 4. Features randomly distributed in *B. avium*

Characteristic	No. of positive strains/total no. of strains examined	Result for type strain 591-77
Oxidase (DPD reagent)[a]	2/28 (w)[b]	−
Alkalinization of *n*-butyramide	14/28 (w)	−
Susceptibility to penicillin G		
2 U/disk	9/28	−
10 U/disk	27/28	+
Production of *N*-glutaryl-L-phenylalanine-2-naphthylamide hydrolase (α-chymotrypsin)[c]	5/8 (w)	w
Growth on the following carbon sources:[d]		
Acetate	3/8 (w)	w
Adipate	6/8 (w)	−
Phenylacetate	1/8 (w)	−
L-Tyrosine	2/8 (w)	w

[a] DPD, *N,N*-Dimethyl-1,4-phenylenediammonium dichloride.
[b] w, Weak positive reaction.
[c] Tested with API ZYM strips.
[d] Tested with API 50AO and API 50AA galleries.

(Tables 2 and 3). There were only nine features for which one or more *B. avium* strains differed (Table 4). In the API auxanographic system, all of the *B. avium* strains investigated grew only on 12 of the 147 carbon sources tested. Moreover, distinct growth was visible only after 2 to 4 days of incubation at 30°C, whereas growth of *B. bronchiseptica* and *Alcaligenes faecalis* was always positive for at least 30 carbon sources after 24 h of incubation.

Comparison of protein electropherograms. The electropherograms of the soluble proteins of 24 *B. avium* strains and 2 other isolates from the respiratory tracts of turkeys (strains 40-81 and P4083) were compared with the electrophoretic patterns of 44 representative strains belonging to the following taxa: *Alcaligenes faecalis, Alcaligenes denitrificans, "Alcaligenes odorans," "Alcaligenes odorans* subsp. *viridans," Achromobacter xylosoxidans, B. bronchiseptica*, and CDC groups IVc-2 and IVe (Table 1). These 44 reference strains were chosen from our electrophoretic data base, which consists of the protein patterns of more than 300 strains belonging or related to the *Alcaligenes-Achromobacter-Bordetella* complex (31; Kersters and De Ley, unpublished data). We included the type strains or the previously designated type strains of the above-mentioned taxa. The reproducibility of the gel electrophoresis technique was checked by comparing the electropherograms of the soluble proteins from two independently grown batches of *B. avium* strain 591-77^T and XX14 cells. The correlation coefficients (*r* values) for eight gels of each strain were greater than 0.95, which is the normal level of reproducibility of the technique (30).

The electrophoretic similarities among the strains studied are shown in Fig. 1 as a sorted and differentially shaded matrix of *r* values. Figure 2 shows normalized photographs of the protein patterns of eight *B. avium* strains, three *B. bronchiseptica* strains, three *Alcaligenes faecalis* strains, two *Alcaligenes denitrificans* strains, two *Achromobacter xylosoxidans* strains and one strain each of CDC groups IVc-2 and IVe. A total of 20 isolates from the respiratory tracts of turkeys formed an extremely homogeneous protein electrophoretic cluster together with 4 strains which were isolated from a chicken, a duck, a goose, and a sharp-tailed munia. The protein patterns of these 24 strains were almost indistinguishable from each other, as they displayed *r* values of 0.90 or higher. We found no significant differences among the electrophoretic patterns of strains isolated from turkeys in the Federal Republic of Germany, Israel, Spain, the United Kingdom, and the United States (Fig. 1 and 2). The protein

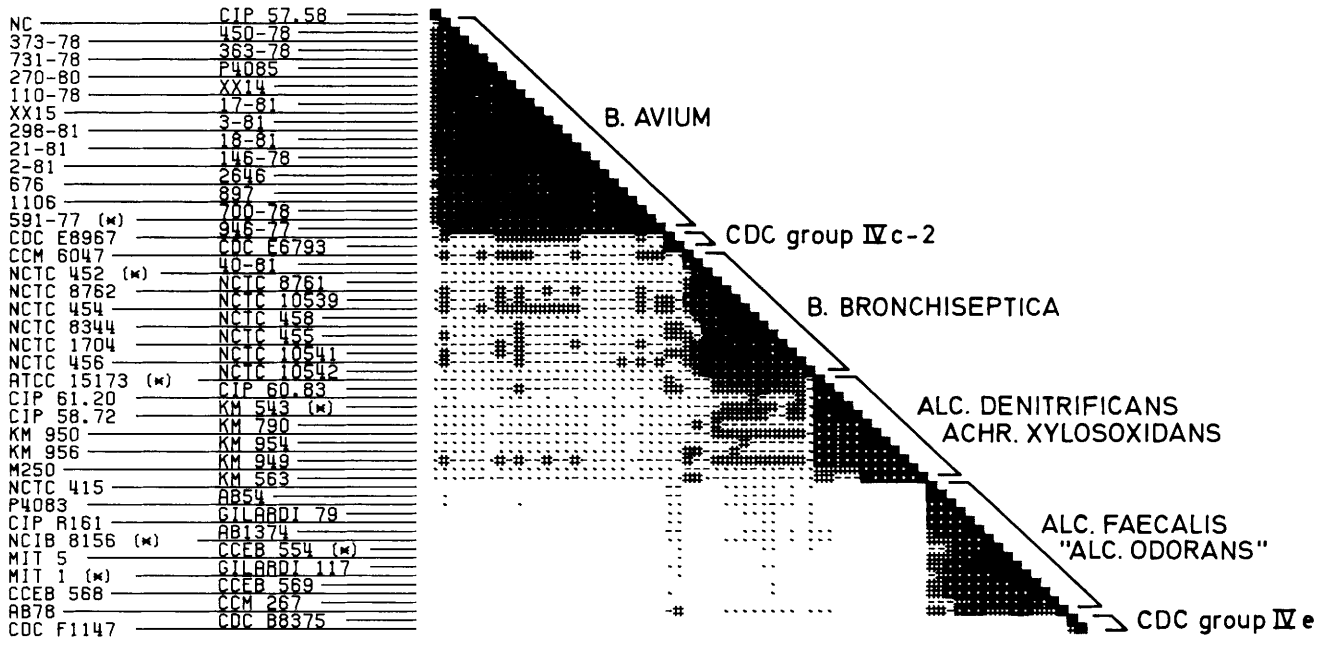

FIG. 1. Sorted and differentially shaded matrix of *r* values calculated from a numerical analysis of protein electropherograms of 24 *B. avium* strains and 46 strains belonging to various groups. The asterisks indicate type strains (60) and previously designated type strains (61).

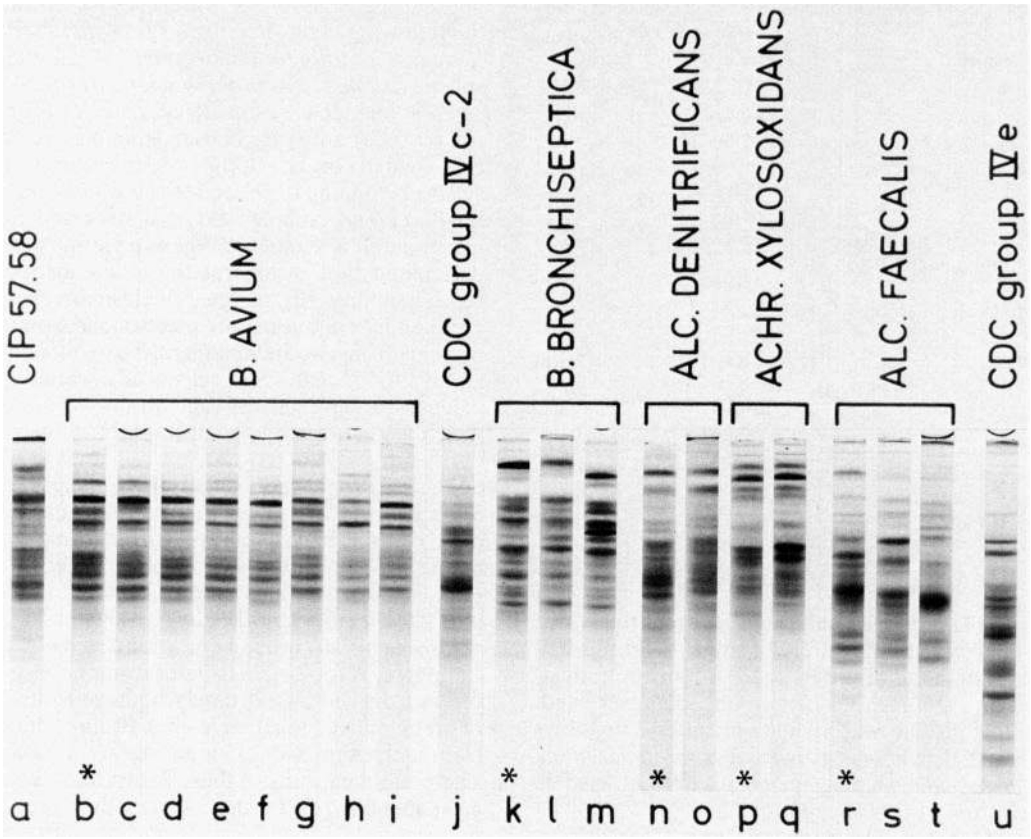

FIG. 2. Normalized electropherograms of the soluble proteins from 8 *B. avium* strains and 13 representative strains belonging to *B. bronchiseptica*, various *Alcaligenes* species, and allied bacteria. Lane a, *Alcaligenes faecalis* CIP 57.58; lane b, *B. avium* 591-77^T (= ATCC 35086^T); lane c, *B. avium* 700-78; lane d, *B. avium* 3-81; lane e, *B. avium* 270-80; lane f, *B. avium* 298-81; lane g, *B. avium* XX14; lane h, *B. avium* NC; lane i, *B. avium* P4085; lane j, CDC group IVc-2 strain CDC E6793; lane k, *B. bronchiseptica* NCTC 452^T; lane l, *B. bronchiseptica* NCTC 10541; lane m, *B. bronchiseptica* 40-81; lane n, *Alcaligenes denitrificans* ATCC 15173^T; lane o, *Alcaligenes denitrificans* CIP 60.83; lane p, *Achromobacter xylosoxidans* KM 543^T; lane q, *Achromobacter xylosoxidans* KM 954; lane r, *Alcaligenes faecalis* NCIB 8156^T; lane s, "*Alcaligenes odorans*" Gilardi 79; lane t, *Alcaligenes faecalis* P4083; lane u, CDC group IVe strain CDC B8375. The asterisks indicate type strains (60).

electropherograms of the *B. avium* strains were clearly distinct from those of all of the other taxa investigated (Fig. 1 and 2). Table 5 lists the average within- and between-taxon similarities of *B. avium* and the clusters formed by various reference strains at an *r* value of 0.9. The average levels of within-taxon similarity of the electropherograms of *B. avium* and *B. bronchiseptica* were very high ($r = 0.95$ and $r = 0.92$, respectively). According to their protein patterns, strains 40-81 and P4083 isolated from turkeys belong to *B. bronchiseptica* and *A. faecalis*, respectively (Fig. 1 and 2). The between-taxon *r* value for *B. avium* and *Alcaligenes faecalis* was 0.52 (Table 5), suggesting that these taxa are not closely

TABLE 5. Within- and between-taxon average similarities of the taxa examined on the basis of numerical comparisons of their protein electropherograms

Taxon or taxa	No. of strains	Avg *r* value[a]						
		Alc. faecalis CIP 57.58	*B. avium*	CDC group IVc-2	*B. bronchiseptica*	*Alc. denitrificans* and *Achr. xylosoxidans*	*Alc. faecalis* and "*Alc. odorans*"	CDC group IVe
Alcaligenes faecalis CIP 57.58	1	—[b]						
B. avium	24	0.86	0.95					
CDC group IVc-2	2	0.76	0.80	0.96				
B. bronchiseptica	14	0.69	0.80	0.80	0.92			
Alcaligenes denitrificans and *Achromobacter xylosoxidans*	12	0.74	0.75	0.77	0.83	0.91		
Alcaligenes faecalis and "*Alcaligenes odorans*"	15	0.46	0.52	0.74	0.62	0.52	0.90	
CDC group IVe	2	−0.14	−0.16	0.14	−0.03	−0.12	0.55	0.89

[a] *r*, Pearson product moment correlation coefficient.
[b] —, Only one strain in a group.

related genetically (see below). When the electropherograms of *B. avium* were compared with those of *B. bronchiseptica*, *Alcaligenes denitrificans*, *Achromobacter xylosoxidans*, and CDC group IVc-2, the average between-taxon *r* values were between 0.75 and 0.80.

Likewise, in sodium dodecyl sulfate-containing gradient gels the protein patterns of the *B. avium* strains were distinct from the protein patterns of the other taxa investigated, including *B. parapertussis* (Fig. 3). However, the overall differences in the electropherograms of *Alcaligenes faecalis* and *B. avium* were more pronounced in the alkaline buffer system used for the native soluble proteins (Fig. 2).

DNA base composition. Table 6 lists the DNA base compositions of eight representative strains of *B. avium*. The values ranged from 61.6 to 62.6 mol% G+C.

Comparison of DNA-rRNA hybrids. To detect the generic and suprageneric relationships of *B. avium*, DNAs from eight representative *B. avium* strains were hybridized with labeled rRNAs from the type strains of *B. bronchiseptica*, *Alcaligenes faecalis*, and *Alcaligenes denitrificans* (Table 6). The rRNA similarities were expressed by the following two parameters: (i) the temperature at which 50% of the hybrid was denatured [$T_{m(e)}$]; and (ii) the percentage of rRNA binding (the amount of rRNA [in micrograms] duplexed to 100 μg of DNA after ribonuclease treatment). Both of these parameters were derived from the thermal denaturation curves of the DNA-rRNA hybrids and were used to construct an rRNA similarity map of hybrids between the ^{3}H-labeled 23S rRNA fraction of *B. bronchiseptica* and DNAs from the bacteria investigated (Fig. 4). Experience has shown that taxonomically $T_{m(e)}$ is the more useful parameter (6, 8, 11, 19). Figure 5 shows the essence of the DNA-rRNA

TABLE 6. DNA base compositions and parameters of the hybrids between DNAs of selected strains and labeled rRNAs of *B. bronchiseptica*, *Alcaligenes denitrificans*, and *Alcaligenes faecalis*

Sequence no.[a]	Organism used for DNA preparation	G+C content (mol%)[b]	*Bordetella bronchiseptica* NCTC 452^T ^{3}H-labeled rRNA		*Alcaligenes denitrificans* ATCC 15173^T ^{14}C-labeled rRNA		*Alcaligenes faecalis* NCIB 8156^T ^{14}C-labeled rRNA	
			$T_{m(e)}$ (°C)	% rRNA binding	$T_{m(e)}$ (°C)	% rRNA binding	$T_{m(e)}$ (°C)	% rRNA binding
1	*Bordetella bronchiseptica* NCTC 452^T	68.9	81.5	0.083	79.5	0.098	75.0	0.083
2	*B. bronchiseptica* NCTC 455	68.9	81.6	0.073	78.5	0.084	75.5	0.068
3	*B. bronchiseptica* CCM 6047	69.0	81.5	0.075	78.6	0.078		
4	*B. parapertussis* NCTC 5952^T [c]	68.6	81.6	0.083	78.7	0.102	75.4	0.082
5	*B. parapertussis* NCTC 7385[c]	68.6	81.4	0.098	79.5	0.091		
6	*B. pertussis* NCTC 10739^T [c]	68.8	81.7	0.101	79.4	0.105	75.5	0.097
7	*B. pertussis* NCTC 8189[c]	68.3	81.6	0.095	79.0	0.113		
8	*B. avium* 591-77^T	61.6	79.0	0.106	77.8	0.116	74.4	0.111
9	*B. avium* 2646	61.7	79.2	0.105	78.7	0.129		
10	*B. avium* XX14	61.9	79.7	0.087	78.4	0.121		
11	*B. avium* 676	61.9	79.7	0.093	78.8	0.128		
12	*B. avium* 363-78	62.2	79.7	0.098	78.6	0.106		
13	*B. avium* 731-78	62.2	79.9	0.102	78.7	0.111		
14	*B. avium* P4085	62.3	79.8	0.113	78.8	0.137		
15	*B. avium* NC	62.6	79.9	0.088	77.8	0.103		
16	*(Alcaligenes faecalis)* CIP 57.58[d]	64.9	80.7	0.085	77.5	0.091	74.5	0.091
17	*Alcaligenes denitrificans* ATCC 15173^T	68.5	79.1	0.063	81.5	0.083	75.0	0.069
18	*Alcaligenes denitrificans* CIP 60.83	66.7	79.2	0.079	80.5	0.088	75.3	0.069
19	*Achromobacter xylosoxidans* KM 543^T	69.2	79.7	0.061	80.5	0.060	76.5	0.053
20	*Achromobacter xylosoxidans* KM 956	67.4	78.7	0.051	80.0	0.068	75.5	0.061
21	*Alcaligenes faecalis* NCIB 8156^T	57.3	76.3	0.067	74.5	0.085	81.5	0.107
22	*Alcaligenes faecalis* AB78	57.9	76.9	0.072	75.0	0.079	81.0	0.089
23	*"Alcaligenes odorans"* Gilardi 117	57.9	76.8	0.100	74.5	0.085	81.0	0.108
24	*Pseudomonas pertucinogena* KM 1319^T	60[e] 67.3	61.5 71.1	0.120 0.063				
25	Group IVc-2 strain CDC E6793	65.9	70.6	0.076				
26	Group IVc-2 strain CDC E8967							
27	Group IVe strain CDC F1147	46.4	70.9	0.094			70.9	0.102
28	Group IVe strain CDC B8375	46.6	71.0	0.100			71.1	0.109

[a] See Fig. 4.

[b] Some of these data have been published previously by De Ley and co-workers; other data are either new values or averages of previous and present measurements.

[c] Inactivated cells were kindly supplied by W. Mannheim, Klinikum der Philipps-Universität Marburg.

[d] Brackets indicate that the strain has been misnamed.

[e] From reference 28.

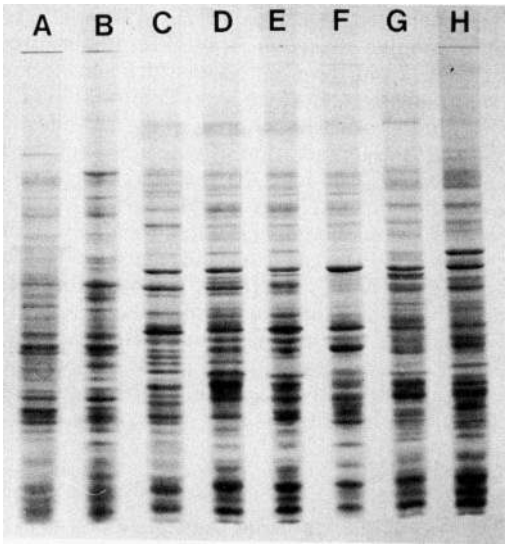

FIG. 3. Comparison of the protein patterns of several *Bordetella*, *Alcaligenes*, and *Achromobacter* strains in a sodium dodecyl sulfate-containing gradient gel. Lane A, *Alcaligenes faecalis* CCM 1052^T (= NCIB 8156^T); lane B, *Alcaligenes faecalis* NCTC 415; lane C, *B. avium* 591-77^T; lane D, *B. bronchiseptica* 40-81; lane E, *B. bronchiseptica* NCTC 452^T; lane F, *B. parapertussis* NCTC 5952^T; lane G, *Alcaligenes denitrificans* NCTC 8582^T (= ATCC 15173^T); lane H, *Achromobacter xylosoxidans* KM 543^T.

hybridization results in a $T_{m(e)}$ dendrogram.

Pathogenicity in turkey poults. All of the *B. avium* strains induced in turkey poults a respiratory disease with the clinical symptoms of turkey coryza (Table 7). *B. avium* was reisolated from the tracheas of all of the inoculated birds, as well as from the majority of the birds exposed by direct contact.

B. bronchiseptica and *Alcaligenes faecalis* strains, as well as turkey isolates 40-81 and P4083, did not produce disease in turkey poults under the experimental conditions used. With the exception of strain 40-81, our attempts to reisolate these organisms from the tracheas of inoculated and contact-exposed birds failed (Table 7).

Serological tests. The results of cross-agglutinin absorption tests showed that the cells of the *B. avium* strains investigated contained six different surface antigenic factors. Type-specific antigens, as well as common surface antigens, were detected, and an antigenic scheme is shown in Table 8. Antisera produced with unheated antigens of three *B. avium* strains (with different surface antigenic structures) did not give cross-agglutination reactions with the *Alcaligenes faecalis* strains used. With *A. denitrificans* NCTC 8582^T a low titer was obtained only with strain 591-77^T antiserum. The titers of cross-reactions between *B. avium* antisera and four *B. bronchiseptica* strains indicated that there are common heat-labile agglutinogens in these two species (Table 9).

As determined by the agar gel precipitation test, the number of precipitation lines distinguishable with the sonicated whole cell antigens of strains 591-77^T, 450-78, and 383-78 varied from four to five when they were tested against the homologous antisera. Three precipitation lines were common to the *B. avium* strains. Strains of *B. avium* and *B. bronchiseptica* had two or three common precipitation lines. One or two precipitation lines were observed between the *B. avium* strains and *Alcaligenes denitrificans* NCTC 8582^T and *Achromobacter xylosoxidans* KM 543^T. There were no reactions between the whole cell antigens of *Alcaligenes faecalis* strains CCM 267 and CCM 1052^T and the *B. avium* strains.

DISCUSSION

The main objective of this study was to reveal the taxonomic status of the avian *Bordetella*-like bacteria that cause coryza (rhinotracheitis) in turkey poults. Polyacrylamide gel electrophoresis of soluble proteins was used to detect genetically nearly identical or highly related strains (31). The relationships of the *Bordetella*-like bacteria to other genera were determined by DNA-rRNA hybridization, which allowed us to detect relationships at the generic and suprageneric levels (8, 10, 19). We have shown previously that the more the midpoint temperature [$T_{m(e)}$ value] of the thermal denaturation curve of a DNA-rRNA hybrid resembles the $T_{m(e)}$ value of the homologous duplex, the more similar the rRNA cistrons are and the more closely the organisms are related (11).

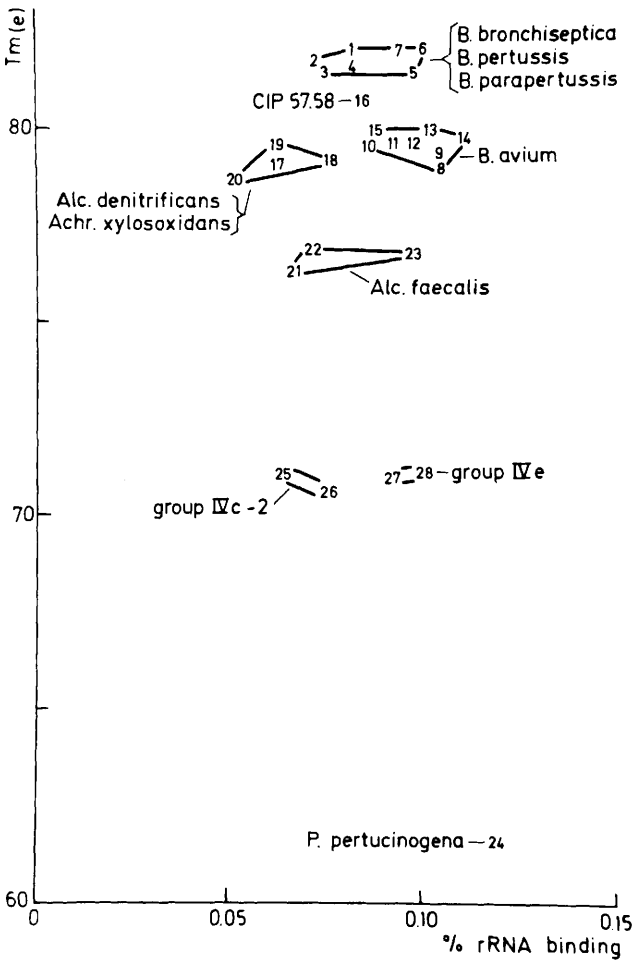

FIG. 4. rRNA similarity map of hybrids between the ^{3}H-labeled 23S rRNA fraction of *B. bronchiseptica* NCTC 452^T and the DNAs from a variety of bacteria, including eight *B. avium* strains. $T_{m(e)}$ values and percentages of rRNA binding were calculated as described in the text. To simplify the drawing, each strain is represented by a sequence number (see Table 6). The positions of all of the strains belonging phenotypically or genetically to the same taxon are joined by lines; these lines are not the ultimate borders, since for some taxa only a limited number of representative strains were investigated.

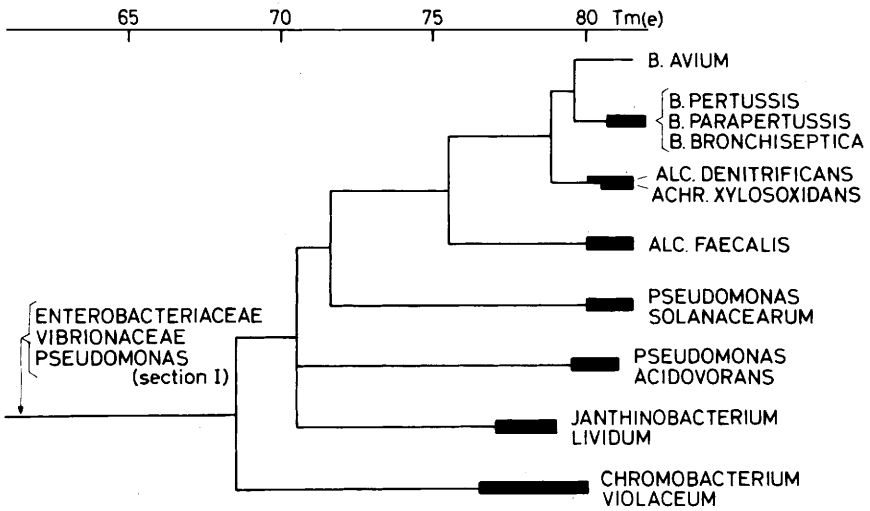

FIG. 5. Levels of $T_{m(e)}$ similarity within the third rRNA superfamily, consisting of *Bordetella*, the authentic *Alcaligenes* strains, *P. solanacearum*, *P. acidovorans*, *Chromobacterium*, and *Janthinobacterium*. The next lower set of rRNA superfamilies is at an average $T_{m(e)}$ of 62°C and consists of the *Enterobacteriaceae*, the *Vibrionaceae*, *Pseudomonas* (section I), and a few other taxa. The data for the graph are from the present study, from references 8, 10, and 12, and from unpublished data of J. De Ley, P. De Vos, M. Gillis, W. Mannheim, and P. Segers. The $T_{m(e)}$ values were clustered by the unweighted average linkage method. The black areas at the ends of most branches represent the ranges of $T_{m(e)}$ values of the reference taxa.

Phenotypic and genetic homogeneity of *B. avium*. The 28 *Bordetella*-like strains for which we propose the name *Bordetella avium* (a'vi.um. L.n. *avis* bird; L. gen. pl. n. *avium* of birds) constitute a phenotypically and genetically homogeneous species. This conclusion was deduced from the great similarities among the strains with respect to their protein electrophoretic patterns (Fig. 1 and 2), their phenotypic (Tables 2 to 4), pathogenic, and serological characteristics (Tables 7 to 9), their DNA G+C contents, and the parameters of the hybrids between the DNAs of eight representative *B. avium* strains and labeled rRNAs of *B. bronchiseptica* and *Alcaligenes denitrificans* (Table 6). Our studies prove that one of the original so-called *Alcaligenes faecalis* strains of Simmons et al. (strain NC, which was isolated from a turkey) (58) belongs to *B. avium*. We also isolated strains from a chicken, a duck, a goose, and a sharp-tailed munia (Table 1). These strains were pathogenic for turkey poults (Table 7) and phenotypically and electrophoretically indistinguishable from the turkey isolates (Fig. 1 and 2). There were no differences in protein patterns among strains belonging to different serotypes or colony types (Table 8) or among strains isolated from different geographic areas (Table 1 and Fig. 1 and 2). The *B. avium* strains were remarkably identical in 95% of the 215 phenotypic features tested. Nutritional and biochemical tests (Table 10), as well as serological and pathogenicity tests (Tables 7 to 9) and comparisons of protein electrophoretic patterns (Fig. 1 and 2) and the parameters of DNA-rRNA hybrids (Fig. 4), indicated that the *B. avium* strains could be differentiated from all of the other taxa investigated. The group I strains of Rimler and Simmons (50) are similar to *B. avium*.

The nutritional requirements of *B. avium* for amino acids and vitamins were not determined. Up to now toxins have not been found. However, it has been shown recently that strains causing rhinotracheitis produce a heat-labile hemagglutinin and a histamine-sensitizing factor in turkey poults and mice (50, 59). The histopathological lesions caused by *B. avium* have been studied (24, 54). The bacteria localize on

TABLE 7. Pathogenicity of *B. avium*, *B. bronchiseptica*, and *Alcaligenes faecalis* for turkey poults

Strain(s) tested	Respiratory symptoms		Recovery of bacteria from trachea	
	Inoculated[a]	Contact exposed[b]	Inoculated[a]	Contact exposed[b]
B. avium (28 strains)	+[c]	+	+	+/−
B. bronchiseptica NCTC 452^T	−	−	−	−
B. bronchiseptica NCTC 454	−	−	−	−
B. bronchiseptica 40-81	−	−	+/−	−/+
Alcaligenes faecalis CCM 1052^T	−	−	−	−
Alcaligenes faecalis NCTC 415	−	−	−	−
Alcaligenes faecalis P4083	−	−	−	−

[a] Five turkey poults were inoculated per strain.

[b] For each strain 5 to 10 non-inoculated turkey poults were placed in direct contact with 5 inoculated poults.

[c] +, Positive result; −, negative result; +/−, more than 50% of the birds were positive; −/+, more than 50% of the birds were negative.

TABLE 8. Surface antigenic factors of eight *B. avium* strains determined by cross-agglutinin absorption tests with antisera absorbed with heated homologous antigen

B. avium strain	Surface antigenic factors	Colony type on blood or VI agar
591-77^T	1, 2, 3, 4	II
946-77	1, 2, 3, 4	II
731-78	1, 2, 3, 4	II
146-78	1, 2, 3, 4	II
700-78	2, 4	II
450-78	1, 2, 6	I
110-78	1, 2, 6	I
383-78	1, 4, 5	I

TABLE 9. Cross-agglutination reactions between the unheated antigens of *B. avium*, *B. bronchiseptica*, *Alcaligenes faecalis*, and *Alcaligenes denitrificans* and antisera prepared against three *B. avium* strains

Agglutination antigens	*B. avium* antiserum against strain:		
	591-77^T	450-78	383-78
B. avium 591-77^T	10,240[a]	2,560	1,280
B. avium 450-78	5,120	40,960	640
B. avium 383-78	320	640	2,560
B. bronchiseptica NCTC 452^T	80	80	320
B. bronchiseptica NCTC 454	80	160	80
B. bronchiseptica NCTC 456	80	80	160
B. bronchiseptica NCTC 458	160	160	40
Alcaligenes faecalis CCM 267	–[b]	–	–
Alcaligenes faecalis NCIB 8156^T	–	–	–
Alcaligenes denitrificans NCTC 8582^T	40	–	–

[a] Reciprocal of agglutination titer.
[b] –, Negative reaction at a serum dilution of >1:10.

and multiply among the cilia of the epithelial cells of the respiratory tracts of turkeys (20, 54). Separate serological studies (K.-H. Hinz and I. Sucker, unpublished data) have shown that *B. avium* strains and *B. bronchiseptica* possess a common heat-stable antigenic determinant (*O*-antigen).

Relationships of *B. avium* to the genus *Bordetella* and other genera. Hybridizations with 23S [^{3}H]rRNA from *B. bronchiseptica* NCTC 452^T revealed a strong similarity among the rRNA cistrons of the three previously recognized *Bordetella* species, *B. pertussis*, *B. parapertussis*, and *B. bronchiseptica* (Table 6 and Fig. 4). DNA-rRNA hybridizations with more than 35 representative *Bordetella* strains (J. De Ley, P. Segers, and W. Mannheim, unpublished data) indicated that these three *Bordetella* species are genetically so closely related that they cannot be differentiated from each other by the parameters of their DNA-rRNA hybrids [$T_{m(e)}$ values ranging from 80.7 to 81.9°C] or by the G+C contents of their DNAs (67.7 to 69.5 mol%) (Table 6 and Fig. 4). These data are in perfect agreement with the high DNA-DNA hybridization values found by Kloos et al. (33) among 15 strains of *B. bronchiseptica*, *B. parapertussis*, and *B. pertussis*. These authors concluded that "the various so-called *Bordetella* species may be reconsidered as representing different subspecies belonging to a single species."

With regard to rRNA cistrons, we found that the following taxa were (in decreasing order) the closest neighbors to the three previously recognized species of the genus *Bordetella*: (i) *Alcaligenes faecalis* strain CIP 57.58; (ii) *B. avium*; and (iii) *Alcaligenes denitrificans* and *Achromobacter xylosoxidans* (66, 67) (Fig. 4 and 5 and Table 6).

When the DNAs of eight representative *B. avium* strains were hybridized with [^{3}H]rRNA from *B. bronchiseptica* NCTC 452^T, all of the hybrids displayed $T_{m(e)}$ values in a narrow range (79.0 to 79.9°C) and rRNA binding values of 0.087 to 0.113% (Table 6 and Fig. 4). The hybrids between *B. avium* DNAs and *Alcaligenes denitrificans* ATCC 15173^T rRNA had somewhat lower $T_{m(e)}$ values (77.8 to 78.8°C) (Table 6), indicating that *B. avium* is more closely related to the genus *Bordetella* than to *Alcaligenes denitrificans* (Fig. 5). Hybridizations with a larger number of strains showed that the rRNA cistrons of *Alcaligenes denitrificans* are indistinguishable from those of *Achromobacter xylosoxidans* (K. Kersters, P. Segers, J. P. Gayral, and J. De Ley, Abstr. 4th Int. Conf. Culture Collections, Brno, Czechoslovakia, P34, 1981; De Ley and Segers, unpublished data). Although the three previously approved *Bordetella* species (60), *B. avium*, and the *Alcaligenes denitrificans-Achromobacter xylosoxidans* group occupied clearly distinct areas on the rRNA similarity map (Fig. 4), a fairly high degree of similarity among the rRNA cistrons of the above-mentioned taxa is evident. Several authors have emphasized that *Alcaligenes denitrificans* and *Alcaligenes faecalis* share some phenotypic features in common with *B. bronchiseptica* (27, 32, 47, 48; Kersters et al., Abstr. 4th Int. Conf. Culture Collections). The DNA base composition of *B. avium* (61.6 to 62.6 mol% G+C [Table 6]) is lower than the values reported for strains belonging to the previously approved *Bordetella* species and the *Alcaligenes denitrificans-Achromobacter xylosoxidans* group (63.9 to 69.8 mol% G+C) (31–33, 68; Kersters et al., Abstr. 4th Int. Conf. Culture Collections; De Ley and Segers, unpublished data). Whether the *Alcaligenes denitrificans-Achromobacter xylosoxidans* group deserves to be incorporated in *Bordetella* will be discussed in a forthcoming paper.

The DNA-rRNA hybridizations indicated that *Alcaligenes faecalis* strain CIP 57.58 (isolated from human sputum) is different from *B. avium*, but is closely related to *Bordetella* and was obviously misnamed (Fig. 4). Kiredjian et al. (32) found that this strain occupied a separate position in their phenotypic and genetic analysis of the genus *Alcaligenes*. Strain CIP 57.58 groups phenotypically at the border of *B. bronchiseptica* (Kersters and Hinz, unpublished data), whereas it clusters at the border of *B. avium* according to its electrophoretic protein pattern (Fig. 1). We are not aware of any other strain which is similar to strain CIP 57.58.

When the DNAs of authentic *Alcaligenes faecalis* strains (i.e., strains resembling type strain NCIB 8156, including "*Alcaligenes odorans*" strains) were hybridized with labeled rRNA of *B. bronchiseptica* NCTC 452^T, they fell into an area with $T_{m(e)}$ values ranging from 76.3 to 76.9°C (Table 6 and Fig. 4). Reciprocal hybridizations between *B. avium* DNA and [^{14}C]rRNAs from *Alcaligenes faecalis* NCIB 8156^T and *Alcaligenes denitrificans* ATCC 15173^T yielded $T_{m(e)}$ values of 74.4 and 77.8 to 78.8°C, respectively (Table 6). Our combined DNA-rRNA hybridization data (Table 6 and Fig. 4 and 5) prove that the *B. avium* strains are clearly different from *Alcaligenes faecalis*. This conclusion was corroborated by comparing phenotypic features (Table 10) and protein electropherograms (Fig. 1 to 3 and Table 5). Therefore, the turkey coryza agent should not be classified as *Alcaligenes faecalis*, as was previously proposed by Simmons et al. (58). It should be noted that the genus *Alcaligenes* as described in *Bergey's Manual of Determinative Bacteriology*, 8th ed. (3), is genetically and phenotypically extremely heterogeneous (29, 31, 32, 45, 68; Kersters et al., Abstr. 4th Int. Conf. Culture Collections). There is much confusion concerning the nomenclature of *Alcaligenes faecalis*; only strains phenotypically and genetically similar to type strain NCIB 8156 (= ATCC 8750 = CCM 1052) should be considered *Alcaligenes faecalis* (32; Kersters et al., Abstr. 4th Int. Conf. Culture Collections). Hendrie et al. (21) considered "*Alcaligenes odorans*" and *Alcaligenes denitrificans* subjective synonyms of *Alcaligenes faecalis*. However, clinical microbiologists often treat *Alcaligenes faecalis* biotype I, *Alcaligenes faecalis* biotype II, and "*Alcaligenes odorans*" as different groups of bacteria. Alkalinization and carbon assimilation tests have indicated that *Alcaligenes faecalis* biotypes I and II are similar to *Alcaligenes denitrificans* and "*Alcaligenes odorans*," respectively (40, 49). The name *Alcaligenes denitrificans* has been recently revived by Rüger and Tan (52).

TABLE 10. Differentiation between *B. pertussis*, *B. parapertussis*, *B. bronchiseptica*, *B. avium*, *Alcaligenes faecalis*,[a] *Alcaligenes denitrificans*, *Achromobacter xylosoxidans*, CDC groups IVc-2 and IVe

Characteristic	*B. pertussis*	*B. parapertussis*	*B. bronchiseptica*	*B. avium*	*Alcaligenes faecalis*[a]	*Alcaligenes denitrificans*	*Achromobacter xylosoxidans*	CDC group IVc-2[b]	CDC group IVe[c]
Parasitic	+[d]	+	+	+	−	−	−		
Localized on respiratory epithelium	+	+	+	+	−	−	−		
Motility	−	−	+	+	+	+	+	+	+[e]
Peritrichous flagella	−	−	+	+	+	+	+	+	+[e]
Growth on:									
MacConkey agar	−	+	+	+	+	+	+	d	d
Bordet Gengou medium (no. of days for appearance of colonies)	3–6	2–3	1–2	1–2					
Browning on peptone agar	−	+	−	d	−	−	−		
Nitrate reduced to nitrite	−	−	+	−	−	+	+	−	+
Nitrate to gas			−	−	−	+	+	−	+
Nitrite to gas			−		+	d	d	−	+
Urease	−	+	+[f]	−	−	d	−	+[f]	+[f]
Phenylalanine deaminase			d	−	−	−	−	−	+
Acid from D-glucose and D-xylose on O/F medium	−	−	−	−	−	−	+	−	−
Carbon sources for growth[g]									
Glucose or xylose		−	−	−	−	−	+	−	−
D-Gluconate		−	−	−	−	+	+	+	−
Acetate		−	+	d	+	+	+	+	+
Glycolate		−	−	−	+	−	−	+	−
Malonate		−	−	−	+	−	−	d	−
Adipate		−	+	d	−	+	+	+	−
Pimelate		−	−	−	−	+	+	+	−
meso-Tartrate		−	d	−	−	+	+	+	−
Itaconate		−	+	−	−	+	+	+	+
Succinate		−	+	+	+	+	+	+	+
Pyruvate, L-proline, or L-glutamate		+	+	+	+	+	+	+	+
Alkalinization of:[h]									
Acetamide or formamide		−	−	+	+	+	+		
Malonamide		−	+	−	+	+	+		
G+C content of DNA (mol%)[i]	67.7–68.9	68.1–69.0	68.2–69.5	61.6–62.6	55.9–59.4	63.9–68.9	66.0–69.8	65.9–67.3[j]	46.4–46[j]

[a] We consider strains phenotypically and genotypically similar to type strain NCIB 8156 members of *Alcaligenes faecalis*; these strains are also very similar to "*Alcaligenes odorans*."

[b] Strains isolated from a variety of human clinical specimens.

[c] Strains isolated mostly from human urine.

[d] +, 90% or more of the strains positive; −, 10% or less of the strains positive; d, 11 to 89% of the strains positive. Unless indicated otherwise, the data were obtained from references 18, 32, 48, 51, 67, and 68 and this study.

[e] Martin et al. (40) found that only 64% of the group IVe strains were motile; these strains possess long lateral and polar flagella (51).

[f] Positive reaction within 4 h.

[g] Tested by using the API 50 auxanographic system (Kersters, unpublished data). The following numbers of strains were tested: *B. parapertussis*, 3; *B. bronchiseptica*, 25; *B. avium*, 8; *Alcaligenes faecalis*, 50; *Alc. denitrificans*, 30; *Achromobacter xylosoxidans*, 50; CDC group IVc-2, 5; CDC group IVe, 4.

[h] Tested by K.-H.H. on Greenwood low-peptone medium (46). The following numbers of strains were tested: *B. parapertussis*, 1; *B. bronchiseptica*, 6; *B. avium*, 28; *Alcaligenes faecalis*, 5; *Alcaligenes denitrificans*, 1; *Achromobacter xylosoxidans*, 1.

[i] Most of these data were obtained from J. De Ley, P. Segers, and W. Mannheim (unpublished data).

[j] Two strains were tested.

The results of DNA-rRNA hybridizations show that neither *B. avium* nor the other *Bordetella* species are related to *Pseudomonas pertucinogena*. We included *P. pertucinogena* (28) because its type strain, strain KM 1319 (= ATCC 190), has been previously assigned to *B. pertussis* (28, 37). Hybridization of the DNA of this strain with [^{14}C]rRNA from *Pseudomonas fluorescens* ATCC 13525^T confirmed that *P. pertucinogena* belongs in the *P. fluorescens* rRNA branch (De Ley, Segers, and Lievens, unpublished data).

Hybridization, DNA base composition, auxanographic, and protein electrophoretic data (Tables 6 and 10 and Fig. 1, 2, and 4) indicate that CDC groups IVc-2 and IVe are not closely related to *B. bronchiseptica*, *B. parapertussis*, *B. pertussis*, *B. avium*, *Alcaligenes faecalis*, *Alcaligenes denitrificans*, or *Achromobacter xylosoxidans*. Reference strains of CDC groups IVc-2 and IVe were included in this study because the correct taxonomic position of these organisms is unknown at this time, and thus their eventual relationship to *B. avium* could not be ruled out a priori. Biochemically, both groups resemble *Alcaligenes* and *B. bronchiseptica* (51).

All of the data given above indicate that the bacteria which cause turkey coryza constitute a new species, namely, *B.*

avium within the genus *Bordetella* as defined by Pittman (47) and Pittman and Wardlaw (48). With regard to rRNA cistron similarities, the genus *Bordetella* belongs to the third rRNA superfamily (sensu De Ley) (6) together with *Alcaligenes faecalis, Alcaligenes denitrificans*, the *Pseudomonas solanacearum* rRNA branch, *Alcaligenes eutrophus*, the *Pseudomonas acidovorans* rRNA branch, *Alcaligenes paradoxus, Derxia, Janthinobacterium*, and *Chromobacterium* (Fig. 5) (8, 10; P. De Vos, P. Segers, and J. De Ley, unpublished data).

We suggest that the genus *Bordetella* should contain the following four species: (i) *B. pertussis* (Bergey, Harrison, Breed, Hammer and Huntoon 1923) Moreno-López 1952, 178 (2, 42); (ii) *B. parapertussis* (Eldering and Kendrick 1938) Moreno-López 1952, 178 (13, 42); (iii) *B. bronchiseptica* (Ferry 1912) Moreno-López 1952, 178 (14, 42); and (iv) *B. avium* sp. nov.

Characteristics that are useful for practical identification of *B. avium* and for differentiation of this species from the other species of the genus *Bordetella* and from more or less related taxa, such as *Alcaligenes faecalis, Alcaligenes denitrificans, Achromobacter xylosoxidans*, and CDC groups IVc-2 and IVe, are shown in Table 10.

Taxonomic position of strains 40-81 and P4083 isolated from turkeys. Protein electropherograms (Fig. 1 and 2) and biochemical and nutritional features proved that strains 40-81 and P4083, which were isolated from the respiratory tracts of turkeys, did not belong to *B. avium* but were authentic members of *B. bronchiseptica* and *Alcaligenes faecalis*, respectively. Neither strain induced rhinotracheitis in turkey poults (Table 7). On blood agar strain P4083 grew as large colonies with raised centers, spreading perimeters and irregular edges and surfaces; these colonies were similar to the colonies of the reference strains of *Alcaligenes faecalis*. On VI agar strain 40-81 grew as colonies of the intermediate type, as described by Bemis et al. (1) for *B. bronchiseptica*.

Description of *Bordetella avium* sp. nov. The description of *B. avium* sp. nov. is based on 28 strains that were isolated from turkeys and from some other birds (Table 1).

The cellular, colonial, biochemical, physiological, and nutritional characteristics are summarized above and in Tables 2 to 4.

B. avium is motile by peritrichous flagella, strictly aerobic, chemoorganotrophic, urease negative, and Kovács oxidase positive; it utilizes glutamic acid, aspartic acid, proline, succinate, and pyruvate. Carbohydrates are not catabolized.

Data on serology and pathogenicity are summarized in Tables 7 to 9. All of the strains which we investigated produce symptoms of rhinotracheitis (coryza) in turkey poults. Pathogenicity for other animals is unknown.

Habitat: isolated from turkeys and a few other birds with symptoms of rhinotracheitis.

G+C content of DNA: 61.6 to 62.6 mol% (thermal denaturation temperature estimation).

Type strain. The type strain is strain Hinz 591-77 (= ATCC 35086), which was isolated by K.-H. Hinz in 1977 from the air sac exudate of a turkey in Hanover, Federal Republic of Germany. The characteristics of the type strain are the same as those given above for the species (Tables 2 to 9). The G+C content of the DNA is 61.6 mol%.

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