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Bordetella hinzii sp. nov., Isolated from Poultry and Humans

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A polyphasic taxonomic study that included DNA-rRNA hybridizations, DNA-DNA hybridizations, DNA base ratio determinations, whole-cell protein and fatty acid analyses, and an examination of classical phenotypic characteristics was performed in order to classify human and veterinary isolates that resemble *Bordetella avium*. Twelve poultry isolates and two human isolates were assigned to a new species, for which we propose the name *Bordetella hinzii*. The position of this organism in the family *Alcaligenaceae* and various genotypic, phenotypic, and chemotaxonomic characteristics are described.

Members of the genus *Bordetella* are well-known pathogens of humans and animals (39). The significance of *Bordetella pertussis*, *Bordetella parapertussis*, and *Bordetella bronchiseptica* as respiratory tract invaders, with whooping cough as the most important infection, is well-known (39). A less familiar member of the genus, *Bordetella avium*, causes respiratory infections in poultry (22). These infections result in high levels of morbidity and are responsible for significant losses in both the turkey and chicken industries (3, 6, 17, 28, 32, 33). Strains of another taxon, which has been referred to as *B. avium*-like, *Alcaligenes faecalis* type II, TC (turkey coryza) bacterium type II, or *Alcaligenes* sp. strain C_2T_2, have also been isolated from respiratory tracts of chickens and turkeys in various parts of the world (1, 3, 19, 20, 31). Although these strains are often isolated from diseased birds, the information available does not provide strong evidence that they are pathogenic (3, 19).

In this study, we performed a polyphasic taxonomic study in order to clarify the taxonomic position of the *B. avium*-like taxon. Below, we show that 12 poultry isolates and 2 human isolates of the *B. avium*-like taxon constitute a novel *Bordetella* species, for which we propose the name *Bordetella hinzii* sp. nov.

MATERIALS AND METHODS

Bacterial strains and growth conditions. All of the strains which we studied and their sources are listed in Table 1. Bacteriological purity was checked by plating and examining living cells, using phase-contrast microscopy and Gram-stained cells.

B. pertussis strains were grown as described by Stainer and Scholte (34). All other strains were grown on Trypticase soy agar (catalog no. 11768; BBL, Becton Dickinson Microbiology Systems, Cockeysville, Md.) and were incubated aerobically at 36 to 37°C unless indicated otherwise.

PAGE of whole-cell proteins. After incubation for 48 h, whole-cell protein extracts were prepared, and sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) was performed as described previously (30). 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).

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 (37).

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Preparation of high-molecular-weight DNA. High-molecular-weight native DNA was prepared as described previously (37).

DNA base compositions. All of the mean guanine-plus-cytosine (G+C) values were determined by the thermal denaturation method, and were calculated as described by De Ley (11).

DNA-DNA hybridization experiments. The degrees of DNA-DNA binding, expressed as percentages, were determined spectrophotometrically by 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% and less indicate that the level of DNA homology is not significant. The total DNA concentration was 48 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 80.5°C.

DNA-rRNA hybridization experiments. In vivo radioactively labelled 23S rRNA from *B. hinzii* LMG 13501^T (T = type strain) was prepared as described previously (36).

Purification of rRNA fractions, fixation of single-stranded DNA on membrane filters, chemical determinations of the amounts of DNA on the filters, saturation hybridization, RNase treatment, and thermostability measurements of the hybrids were performed as described by Van Landschoot and De Ley (38). Each DNA-rRNA hybrid was characterized by its melting temperature of elution [$T_{m(e)}$], the temperature at which 50% of the hybrid was denatured. The higher the $T_{m(e)}$ of a heterologous hybrid, the closer the two strains are related. The $T_{m(e)}$ values obtained from reciprocal hybridization experiments in which all strains belonging to each rRNA branch were compared were used to calculate the average linkage level between each pair of rRNA branches.

Phenotypic tests. Strains were grown on brain heart infusion base (catalog no. 11037; BBL) supplemented with 5% sheep blood for maintenance of cultures and preparation of inocula for biochemical tests. The other media used are described below. Blood agar plates were incubated in air containing 5% CO_2; other media were incubated aerobically. The incubation temperature was 37°C unless indicated otherwise.

The gram stain reaction was assessed by using cells obtained from cultures incubated on blood agar for 16 h. The occurrence and distribution of flagella were determined by electron microscopy, using a phosphotungstic acid negative stain on cultures grown overnight in nutrient broth incubated at room temperature. Oxidase activity was tested by using 18-h-old cultures and a Marion oxidase dropper (Marion Scientific). Alkalinization of amides and organic salts was determined by using a modified Greenwood low-peptone medium (29), as previously described (4). Briefly, the basal medium contained 0.1 g of $(NH_4)_2HPO_4$, 0.02 g of KCl, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.05 g of yeast extract, 0.05 g of Casitone, 0.02 g of glucose, 1.5 g of agar, 2.5 ml of a 0.4% aqueous solution of bromthymol blue, and 100 ml of distilled water. The basal medium was adjusted to pH 6.5 before sterilization and autoclaving. The amides and organic salts were sterilized by filtration and were added to the basal medium to final concentrations of 1% (wt/vol). The completed test media were dispensed as 3-ml slants in cotton wool-plugged glass tubes (110 by 13 mm). Growth from a 16-h culture on blood agar was heavily streaked onto the slant of medium in each tube. The substrates used were acetamide, disodium adipate, glycine, malonamide, sodium malonate, sodium valerate, and propionamide. A dark blue color, which indicated an alkaline pH, was recorded as positive. Standard methods were used for all other biochemical tests (10). The urease test medium used was a liquid version of Christensen's medium (catalog no. CM71; Oxoid). Unless otherwise indicated, the inoculated test media were observed daily, and the final reading was obtained after 5 days.

The API 20NE MicroTest system was used according to the recommendations

TABLE 1. Strains studied

Strain[a]	Taxon	Original designation(s)[a]	Depositor(s)[a,b]	Source (if known)
LMG 1229^T	*Alcaligenes faecalis*	NCIB 8156^T, ATCC 8750^T	NCIB, ATCC	
LMG 1230	*Alcaligenes faecalis*	CCEB 554	CCEB	Feces (Czechoslovakia)
LMG 1861	*Alcaligenes piechaudii*	NCMB 1051	Torry Research Station	Soil
LMG 1873^T	*Alcaligenes piechaudii*	CIP 60.75^T	CIP	Pharynx
LMG 1231^T	*Alcaligenes xylosoxidans* subsp. *denitrificans*	ATCC 15173^T	ATCC	Soil
LMG 1863^T	*Alcaligenes xylosoxidans* subsp. *xylosoxidans*	ATCC 27061^T	E. Yabuuchi	Human ear discharge (Japan)
LMG 10973	*Bordetella avium*	Hommez 15		Trachea of broiler chicken with slight respiratory signs and tracheitis (1989, Torhout, Belgium)
LMG 10974	*Bordetella avium*	Hommez 16		Chicken with coryza (Torhout, Belgium)
LMG 10975	*Bordetella avium*	Hommez 17		Trachea of chicken with coryza, dyspnea, lung congestion, and slight tracheitis (1988, Torhout, Belgium)
LMG 10976	*Bordetella avium*	Hommez 18		Spleen of chicken with Marek's disease (1989, Torhout, Belgium)
LMG 10977	*Bordetella avium*	Hommez 19		Trachea of broiler chicken with coryza-like symptoms and slight tracheitis (1988, Torhout, Belgium)
LMG 10978	*Bordetella avium*	Hommez 20		Trachea of broiler chicken with slight tracheitis and polyserositis (1988, Torhout, Belgium)
LMG 1851	*Bordetella avium*	Hinz 363-78	K.-H. Hinz	Turkey with turkey coryza, respiratory tract (Germany)
LMG 1852^T	*Bordetella avium*	Hinz 591-77^T, ATCC 35086^T	K.-H. Hinz, ATCC	Turkey, air sac exudate (Germany)
LMG 1854	*Bordetella avium*	Rimler P-4085	K.-H. Hinz	Turkey (United States)
LMG 1858	*Bordetella avium*	Simmons strain NC	K.-H. Hinz	Turkey (United States)
LMG 3524	*Bordetella avium*	Hinz 946-77	K.-H. Hinz	Turkey with turkey coryza, lung (Germany)
LMG 3549	*Bordetella avium*	Hinz 2-81	K.-H. Hinz	Turkey (United Kingdom)
LMG 3557	*Bordetella avium*	Hinz 298-81	K.-H. Hinz	Duck, trachea (Germany)
LMG 1232^T	*Bordetella bronchiseptica*	NCTC 452^T, ATCC 19395^T	NCTC, ATCC	Dog, lung
LMG 3521	*Bordetella bronchiseptica*	Hinz 40-81	K.-H. Hinz	Turkey, trachea (Germany)
LMG 10979	*Bordetella hinzii*	Hommez 6, TC102		Chicken with slight tracheitis (Torhout, Belgium)
LMG 10980	*Bordetella hinzii*	Hommez 7, TC103		Chicken with slight tracheitis (Torhout, Belgium)
LMG 13494	*Bordetella hinzii*	TC18		Chicken, sinus (Australia)
LMG 13495	*Bordetella hinzii*	TC27		Chicken, trachea (Australia)
LMG 13496	*Bordetella hinzii*	TC32		Chicken, trachea (Australia)
LMG 13497	*Bordetella hinzii*	TC33		Chicken, sinus (Australia)
LMG 13498	*Bordetella hinzii*	TC36		Chicken, trachea (Australia)
LMG 13499	*Bordetella hinzii*	TC38		Chicken, trachea (Australia)
LMG 13500	*Bordetella hinzii*	TC52		Chicken, trachea (Australia)
LMG 13501^T	*Bordetella hinzii*	TC58^T		Chicken, trachea (Australia)
LMG 13504	*Bordetella hinzii*	TC81		Chicken, trachea (Australia)
LMG 13505	*Bordetella hinzii*	TC82		Chicken, trachea (Australia)
LMG 14052	*Bordetella hinzii*	TC104		AIDS patient (United States)
LMG 1872	*Bordetella hinzii*	CIP 57.58, TC101	CIP	Sputum (France)
LMG 1816	*Bordetella parapertussis*	NCTC 7385	NCTC	
	Bordetella pertussis	NIH L4		
LMG 13506	*Bordetella* sp.	1779		Chronic otitis media, human (Germany)

[a] ATCC, American Type Culture Collection, Rockville, Md.; CCEB, Culture Collection of Entomogenous Bacteria, Department of Insect Pathology, Institute of Entomology, Prague, Czechoslovakia; CIP, Collection bactérienne de l'Institut Pasteur, Paris, France; LMG, Laboratorium Microbiologie Gent Culture Collection, Universiteit Gent, Ghent, Belgium; NCIB, National Collection of Industrial Bacteria, NCIMB, Ltd., Aberdeen, Scotland; NCMB, National Collection of Marine Bacteria, NCIMB, Ltd., Aberdeen, Scotland; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, United Kingdom; NIH, National Institutes of Health, Bethesda, Md.

[b] Our isolate if not specified.

of the manufacturer (bioMérieux, La Balme-les-Grottes, Montalieu-Vercieu, France).

RESULTS

PAGE of whole-cell proteins. Duplicate protein extracts were prepared to check the reproducibility of the growth conditions and the preparation of the extracts. The levels of correlation (r) between duplicate protein patterns were $\geq$0.94.

The protein profiles and the corresponding dendrogram obtained after numerical analysis are shown in Fig. 1. Seven stable clusters and four strains that occupied separate positions were differentiated. Clusters I, II, and V comprised *Alcaligenes piechaudii*, *A. faecalis*, and *B. bronchiseptica* reference strains that grouped at r values of 0.93, 0.92, and 0.84, respectively. Clusters III and IV contained *B. hinzii* strains that grouped at r values of more than 0.84. Clusters VI and VII contained *B. avium* strains that grouped at r values of more than 0.83 and 0.89, respectively. The *Alcaligenes xylosoxidans* subsp. *xylosoxidans* and *A. xylosoxidans* subsp. *denitrificans* reference strains and strains LMG 1872 (*B. hinzii*) and LMG 13506 occupied separate positions on the dendrogram.

The major difference between the protein profiles of the *B. hinzii* strains belonging to clusters III and IV was the position of a prominent protein band that had a molecular weight of approximately 33,000 (Fig. 1). In addition, the position of a second protein band (molecular weight, approximately 23,000

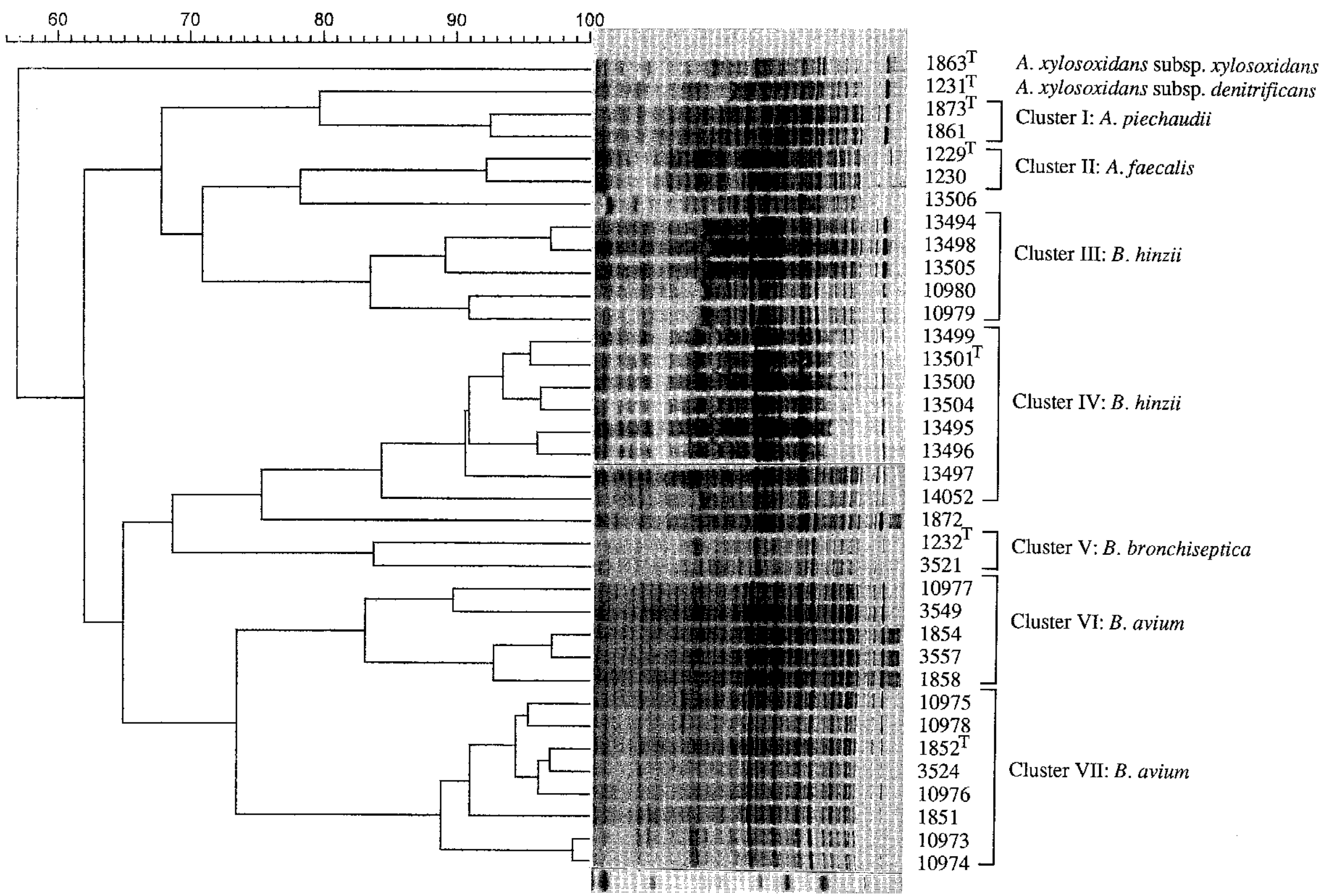

FIG. 1. Electrophoretic protein profiles of the strains studied. All strain numbers are Laboratorium Microbiologie Gent (LMG) numbers. The dendrogram was derived from an unweighed pair group average linkage analysis of the r values obtained from comparisons of the protein patterns of the strains. The molecular weight markers used (lane at the bottom) were (from left to right) lysozyme (molecular weight, 14,500), trypsin inhibitor (20,100), trypsinogen (24,000), glyceraldehyde-3-phosphate dehydrogenase (36,000), egg albumin (45,000), bovine albumin (66,000), and β-galactosidase (116,000).

[Fig. 1]) differed in cluster III and IV strains. The protein pattern of *B. hinzii* LMG 1872 was distorted, although it was virtually identical to the protein patterns of the cluster III strains. A second protein extract was prepared, but the same distortion was found (data not shown). In general, the differences between the *B. avium* strains belonging to clusters VI and VII were restricted to differences in one high-molecular-weight protein band which was prominent in cluster VI strains but less prominent in cluster VII strains (molecular weight, more than 116,000 [Fig. 1]).

Cellular fatty acid analysis. The major fatty acid components of all of the strains examined were 16:0, 17:0 cyclo, and summed feature 3. Summed feature 3 comprised two fatty acids which could not be distinguished by the Microbial Identification System, 16:1 iso I and 14:0 3OH. Branched-chain fatty acids have not been reported in *Bordetella* or *Alcaligenes* strains, or they are present only in trace amounts in some strains (20). Therefore, the peak designated summed feature 3 probably corresponds to 14:0 3OH. The major fatty acids accounted for 75% or more of the total fatty acid contents of all of the strains studied and were not useful for differentiating the various taxa.

The average fatty acid compositions of all species were calculated, and these compositions are shown in Table 2.

DNA base compositions. All of the DNA base ratios determined are shown in Table 3. The *B. hinzii* strains had DNA base ratios of 65 to 67 mol%, values which are similar to the *B. pertussis*, *B. parapertussis*, and *B. bronchiseptica* values. *B. avium* remained the most aberrant member of the genus *Bordetella*, with a G+C content of 61 to 62 mol%.

DNA-DNA hybridization experiments. The results of the DNA-DNA hybridization study indicated that the *B. hinzii* strains belonging to electrophoretic clusters III and IV and strain LMG 1872 constitute a homogeneous genospecies (DNA binding values, ≥67%) (Table 3). No significant DNA binding values were obtained when these strains were hybridized with strain LMG 13506 or reference strains of other *Bordetella* or *Alcaligenes* species.

The *B. avium* strains belonging to clusters VI and VII were highly related (DNA binding value, 95%).

B. pertussis, *B. parapertussis*, and *B. bronchiseptica* constituted a single, homogeneous genospecies (DNA binding values, >76%). These data confirmed the findings of Kloos et al. (25) and indicated that these three species can be considered subtypes of a single species (25).

Reference strains of all taxa were cross-hybridized, and no significant DNA binding values were obtained (except when *B. pertussis*, *B. parapertussis*, and *B. bronchiseptica* were used [see above]).

DNA-rRNA hybridization experiments. The DNA-rRNA hybridization results are shown in Table 4. The average linkage levels in the dendrogram in Fig. 2 are based on the $T_{m(e)}$ values which we obtained and on previously published data (13).

Phenotypic tests. All *B. hinzii* strains, together with the type strains of *B. avium*, *B. bronchiseptica*, and *A. faecalis*, were examined for phenotypic characteristics. All of the strains were gram-negative rod-shaped organisms that were motile by means of peritrichous flagella. All strains produced catalase and oxidase and gave an alkaline reaction in the oxidative-fermentative test.

When the API 20NE MicroTest system was used, the following characteristics were always negative: reduction of nitrate (except *B. bronchiseptica* ATCC 19395^T), production of indole, production of acid from glucose, urease activity (except *B. bronchiseptica* ATCC 19395^T), arginine dihydrolase and β-galactosidase activities, liquefaction of gelatin, hydrolysis of

TABLE 2. Average fatty acid compositions of the strains studied

Taxon	% of[a]:														
	12:0	12:0 2OH	14:0	14:0 2OH	16:1 *cis* 9	16:0	ECL 16.089[b]	17:0 cyclo	16:0 2OH	Unknown 17.749	18:0	Unknown 18.439	19:0 cyclo C11-12	SF3[c]	18:1[d]
B. hinzii (14 strains)	Tr	3.4 ± 0.4	1.3 ± 0.5	4.6 ± 0.5	1.4 ± 0.5	32.4 ± 2.2	Tr	32.4 ± 1.4	2.5 ± 0.7	Tr	4.0 ± 1.9	1.5 ± 0.3	1.3 ± 0.6	10.7 ± 0.9	Tr
B. avium (14 strains)	Tr	3.0 ± 0.9	1.8 ± 0.7	3.8 ± 0.9	Tr	34.7 ± 3.6	Tr	35.3 ± 2.9	1.5 ± 1.4	1.1 ± 0.4	3.7 ± 1.6	1.7 ± 0.9	1.2 ± 0.9	9.1 ± 2.3	ND
Bordetella sp. strain LMG 13506	ND	2.5	1.3	4.4	ND	36.1	1.4	31.6	3.3	1.2	4.4	2.6	1.4	9.1	ND
B. bronchiseptica (2 strains)	Tr	3.5 ± 1.1	6.7 ± 0.4	ND	1.8 ± 0.3	36.3 ± 4.8	Tr	34.0 ± 1.8	ND	Tr	1.8 ± 0.5	1.1 ± 0.3	1.1 ± 0.9	9.3 ± 2.6	Tr
A. faecalis (2 strains)	2.1 ± 0.9	3.4 ± 0.6	1.7 ± 0.1	ND	2.9 ± 0.8	30.0 ± 4.0	Tr	33.8 ± 1.6	ND	Tr	Tr	1.1 ± 0.2	2.1 ± 0.2	13.6 ± 2.3	3.9 ± 1.8
A. piechaudii (2 strains)	Tr	3.2 ± 0.1	6.1 ± 0.1	ND	3.1 ± 0.2	31.7 ± 1.0	Tr	35.0 ± 0.9	1.0 ± 0.2	Tr	1.3 ± 0.1	1.2 ± 0.0	1.0 ± 0.1	9.1 ± 0.5	3.7 ± 0.5
A. xylosoxidans subsp. *xylosoxidans* LMG 1863^T	Tr	3.9	1.1	4.8	5.8	35.2	Tr	28.4	1.5	Tr	1.5	Tr	1.1	11.2	Tr
A. xylosoxidans subsp. *denitrificans* LMG 1231^T	1.6	2.9	6.4	ND	4.3	35.2	Tr	29.4	1.0	Tr	1.2	Tr	1.0	10.2	2.4

[a] The fatty acids which account for less than 1% of the total fatty acids in all of the strains studied are not included. Therefore, the percentages may not add up to 100%. ND, not detected; Tr, trace (less than 1%).
[b] ECL, equivalent chain length. The identity of the fatty acid is not known.
[c] SF3, summed feature 3 (16:1 iso I or 14:0 3OH or both).
[d] This peak is summed feature 7, which comprises three isomers of 18:1 (18:1 *cis* 11, 18:1 *trans* 9, and 18:1 *trans* 6).

TABLE 3. DNA-DNA hybridization results and DNA base compositions of *Bordetella* and *Alcaligenes* strains

Taxon	Strain	G+C content (mol%)	% DNA-DNA homology[a]															
			B. pertussi NIH L4	*B. parapertussis* LMG 1816	*B. bronchiseptica* LMG 1232^T	*B. avium* LMG 1852^T	*B. avium* LMG 3549	*B. hinzii* LMG 13501^T	*B. hinzii* LMG 13495	*B. hinzii* LMG 10979	*B. hinzii* LMG 13505	*B. hinzii* LMG 14052	*B. hinzii* LMG 1872	*Bordetella* sp. strain LMG 13506	*A. xylosoxidans* subsp. *xylosoxidans* LMG 1863^T	*A. xylosoxidans* subsp. *denitrificans* LMG 1231^T	*A. faecalis* LMG 1229^T	*A. piechaudii* LMG 1873^T
B. pertussis	NIH L4	67	100															
B. parapertussis	LMG 1816	69	76	100														
B. bronchiseptica	LMG 1232^T	69	83	91	100													
B. avium	LMG 1852^T	62	22	16		100												
	LMG 3549	61				95	100											
B. hinzii	LMG 13501^T	66						100										
	LMG 13495	67						95	100									
	LMG 10979	67	18	19	15	13		91		100								
	LMG 13505	67							89	95	100							
	LMG 14052	67						89		89		100						
	LMG 1872	65			10	27		78		67		71	100					
Bordetella sp.	LMG 13506	65			30	3		15		22				100				
A. xylosoxidans subsp. *xylosoxidans*	LMG 1863^T	69						16		21				21	100			
A. xylosoxidans subsp. *denitrificans*	LMG 1231^T	67						8		18				19	26	100		
A. faecalis	LMG 1229^T	57						11		6				9	8	12	100	
A. piechaudii	LMG 1873^T	65						9		6				20	19	24	3	100

[a] Each value is the average of the levels of binding obtained in at least two hybridization experiments.

TABLE 4. $T_{m(e)}$ values of the DNA-rRNA hybrids obtained with 23S rRNA of *B. hinzii* LMG 13501^T

Source of DNA	$T_{m(e)}$ (°C)
B. avium LMG 1851	81.0
B. avium LMG 3549	80.3
B. bronchiseptica LMG 1232^T	81.2
B. parapertussis LMG 1816	80.7
B. pertussis NIH L4	81.1
B. hinzii LMG 1872	81.6
B. hinzii LMG 13501^T	81.8
B. hinzii LMG 10979	81.8
B. hinzii LMG 14052	81.8
Bordetella sp. strain LMG 13506	78.3
A. faecalis LMG 1229^T	74.3
A. xylosoxidans subsp. *xylosoxidans* LMG 1863^T	80.3
A. xylosoxidans subsp. *denitrificans* LMG 1231^T	79.7
A. piechaudii LMG 1873^T	80.6

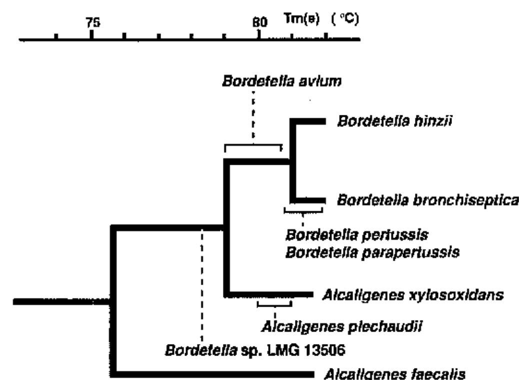

FIG. 2. Simplified rRNA cistron similarity dendrogram of the family *Alcaligenaceae*. The dashed lines show the positions of taxa for which no labeled rRNAs were prepared. The average linkage levels were calculated by using the $T_{m(e)}$ values shown in Table 4 and previously published values (13).

esculin, and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, maltose, and D-gluconate. Assimilation of citrate and phenyl acetate and oxidase activity were always positive.

All of the remaining characteristics were strain or taxon dependent (Table 5).

DISCUSSION

We performed a polyphasic taxonomic study to clarify the phylogenetic position of a taxon previously referred to as *A. faecalis* type II, TC (turkey coryza) bacterium type II, *Alcaligenes* sp. strain C_2T_2 or *B. avium*-like (1, 3, 19, 20, 31). In this study, strains of *B. avium*, *B. pertussis*, *B. parapertussis*, *B. bronchiseptica*, *A. faecalis*, *A. piechaudii*, *A. xylosoxidans* subsp. *xylosoxidans*, and *A. xylosoxidans* subsp. *denitrificans* were included as reference strains because members of the genera *Bordetella* and *Alcaligenes* are closely interrelated and hard to differentiate on the basis of genotypic and phenotypic criteria (13, 22, 23). Three unclassified human clinical isolates were considered as well (Table 1).

The results of our study allowed us to assign 12 veterinary isolates and 2 human clinical strains to a new species, *B. hinzii*.

Species delineation by whole-cell protein electrophoresis. SDS-PAGE of whole-cell proteins and numerical comparisons of the protein patterns are often used to compare large numbers of bacteria and to group closely related strains (30, 37). A visual comparison of the protein profiles of all of the strains which we studied corroborated the initial phenotypic identification of six Belgian chicken isolates (LMG 10973 through LMG 10978) as *B. avium* (18). Although the *B. avium* strains were subdivided into two clusters on the basis of the results of a numerical analysis of their protein patterns, DNA-DNA hybridization data confirmed that these strains constituted a genotypically homogeneous species (Table 3), as determined previously in a DNA-rRNA hybridization study (22).

The 2 remaining Belgian field isolates (LMG 10979 and LMG 10980), the 10 Australian strains of the *B. avium*-like taxon, and 2 unclassified human clinical isolates produced very similar protein profiles (Fig. 1). As explained above, subdivision of these strains into two clusters on the basis of the results of a numerical analysis of their protein patterns was primarily due to a single dense protein band with a variable molecular weight of about 33,000. Similar variable dense protein bands that influence the clustering sequences of strains within species have been reported in several other genera and can be omitted from numerical analyses to enhance species differentiation (reviewed in reference 9). As deduced from the very similar protein contents and confirmed by DNA-DNA hybridization data, the strains belonging to the two clusters and strain LMG 1872 constitute a homogeneous species (DNA binding values, >67%).

Taxonomic position of *B. hinzii*. Figure 2 shows the phylogenetic position of *B. hinzii* within the family *Alcaligenaceae*, as determined by DNA-rRNA hybridization. The *B. hinzii* strains constitute a separate rRNA branch, and *B. pertussis*, *B. parapertussis*, and *B. bronchiseptica* are its closest neighbors [differences in $T_{m(e)}$, up to 1°C] (Table 4). *B. avium* and members of the *A. xylosoxidans* rRNA branch (*A. xylosoxidans* and *A. piechaudii*) are somewhat further removed [differences in $T_{m(e)}$, up to 2°C] (Fig. 2), which confirms and extends findings obtained by comparative analysis of 23S rRNA sequences (27). DNA-DNA hybridization studies did not reveal significant DNA binding with *B. pertussis*, *B. parapertussis*, and *B. bronchiseptica* or with other members of the *Alcaligenaceae* (Table 3).

Chemotaxonomic characteristics of *B. hinzii*. Several studies have focused on chemotaxonomic characteristics of *B. avium* and related bacteria (15, 20, 26). In this study, the major fatty acid components of all of the strains examined were 16:0, 17:0 cyclo, and summed feature 3 (probably 14:0 3OH) (Table 2); these findings generally corroborate previously published data. Minor quantitative differences have been reported between *B. avium* and *B. hinzii* strains when the organisms were cultivated under strictly defined conditions (20, 26). These differences could not be detected under the conditions which we used, and hence, we could not differentiate *B. avium* from *B. hinzii* (Table 2). Furthermore, we could not differentiate *A. piechaudii* from *A. xylosoxidans* subsp. *denitrificans* (Table 2). All other species or subspecies could be differentiated on the basis of qualitative or quantitative differences (or both) in minor components. Strains could not be differentiated on the generic level, which further substantiates the genotypic interrelatedness of the genera *Bordetella* and *Alcaligenes* (13).

In addition, *B. hinzii*, *B. avium*, *B. pertussis*, *B. bronchiseptica*, *A. faecalis*, and *A. xylosoxidans* subsp. *denitrificans* have all been reported to contain ubiquinone 8 as their major respiratory quinone along with trace amounts of ubiquinone 9 (7, 15, 35).

Phenotypic characterization of *B. hinzii*. Phenotypic studies of *B. hinzii* strains have always focused on organisms derived

TABLE 5. Phenotypic characteristics of the strains examined

Characteristic	*B. hinzii*			*B. avium* ATCC 35086[T]	*B. bronchiseptica* ATCC 19395[T]	*A. faecalis* ATCC 8750[T]
	General reaction result	Reaction of LMG 13501[T]	Aberrant strains			
Urease activity (in Christensen's medium)	V (2/14)[a]	−	LMG 13497, LMG 13499	−	+	−
Urease activity (in API 20NE system)	−	−		−	+	−
Nitrate reduction	−	−		−	+	−
Alkali production from:						
Acetamide	+	+		+	−	−
Adipate	+	+		+	+	−
Glycine	V (9/14)	+	LMG 13494, LMG 13497, LMG 13498, LMG 13499, LMG 13505	−	+	+
Malonamide	+	+		−	+	+
Malonate	+	+		−	+	+
Propionamide	+	+		+	+	+
Valerate	+	+		−	+	+
Assimilation of caprate	V (12/14)	+	LMG 1872, LMG 10980	−	−	+
Assimilation of adipate	+	+		+	+	−
Assimilation of L-malate	+	+		+	−	+
API 20NE profile	0000077 (12/14)	0000077	LMG 1872, LMG 10980[b]	0000067	1200027	0000057

[a] The numbers in parentheses are the numbers of strains that are positive for the characteristic. +, present in all strains; −, absent in all strains; V, strain-dependent reaction result.
[b] The API 20NE profile of these strains is 0000067.

from veterinary sources, with the main objective being the differentiation of *B. hinzii* from *B. avium* (1, 3, 19, 31). *B. hinzii* strains have been reported to be different from *B. avium* strains in the following characteristics: *B. hinzii* strains are not able to agglutinate erythrocytes from chickens, turkeys, humans, and guinea pigs and are able to grow in the presence of 6.5% NaCl, in a minimal essential medium, in Simmons citrate agar, and in the presence of cetrimide (1, 19, 31). In addition, *B. hinzii* strains alkalinize considerably more substrates than *B. avium* strains and decarboxylate histidine (1, 19, 31). However, often the inoculum size and method used may influence the results of tests, as has been demonstrated for several of these characteristics (1, 5, 16).

We evaluated a number of classical phenotypic tests and the API 20NE MicroTest system for their ability to differentiate *B. avium*, *B. hinzii*, *B. bronchiseptica*, and *A. faecalis*. Alkali production from malonamide, malonate, and valerate clearly differentiated *B. hinzii* from *B. avium* (Table 5). *B. hinzii* strains also differed from *B. avium* in the API 20NE tests by their ability to assimilate caprate (Table 5). However, two aberrant strains were incorrectly identified as *B. avium* (Table 5). Other characteristics that can be used to differentiate *B. hinzii*, *B. avium*, *B. bronchiseptica*, and *A. faecalis* are shown in Table 5.

The absence of motility, the absence of citrate utilization, and fastidious growth characteristics readily differentiate *B. pertussis* from *B. hinzii* (39). In addition, it is unlikely that the various pertussis virulence factors (39) are present in *B. hinzii*. The absence of motility, the absence of oxidase acitivity, the presence of urease acitivity, and browning of the growth medium differentiate *B. parapertussis* from *B. hinzii* (39).

Clinical significance of field isolates. The pathological signs associated with the six field isolates identified as *B. avium* (LMG 10973 through LMG 10978) included coryza-like symptoms, tracheitis, and polyserositis and support the hypothesis that *B. avium* is pathogenic in chickens.

Twelve *B. hinzii* strains were obtained from the usual sources (i.e., the respiratory tracts of turkeys or chickens) (Table 1). The available clinical histories of these organisms did not suggest that they are pathogenic. The inclusion of human clinical isolates in this taxon has not been reported previously. Strain LMG 1872 was isolated from sputum and was originally identified as *A. faecalis* (24). Several taxonomic studies demonstrated that this isolate belongs to the *Bordetella-Alcaligenes* group but failed to classify it properly (13, 22, 24). No data on the clinical significance of this strain are extant. A second human isolate, LMG 14052, was isolated from a human immunodeficiency virus-infected male (8). This strain grew in all four of four blood cultures drawn from the patient, and this episode of bacteremia required hospitalization. Intravenous antibiotic therapy was successful, but the patient did not have any evidence of respiratory illness or a history of exposure to birds. This case suggests that *B. hinzii* may be a previously unidentified human pathogen that does not necessarily cause disease via the respiratory tract or exist only in birds.

Description of *Bordetella hinzii* sp. nov. *Bordetella hinzii* (hin′zi.i. N. L. gen. n. *hinzii* [of Hinz], named in honor of K.-H. Hinz, a German microbiologist who has contributed much to our knowledge of avian isolates belonging to the genus *Bordetella*). *B. hinzii* cells are gram-negative, nonsporulating rods that are motile by means of peritrichous flagella. Two distinct colony types occur. Some strains produce round, raised, glistening, greyish colonies about 2 mm in diameter following 48 h of incubation at 37°C in air containing 5% CO_2. Under the same conditions other strains produce flat, dry, crinkled colonies that are up to 5 mm in diameter. Nitrates are not reduced, and oxidase and catalase activities are present. Urease activity in Christensen's medium is strain dependent. Alkali is produced from the following substrates: acetamide, adipate, malonamide, malonate, valerate, and propionamide. Production of alkali from glycine is strain dependent. As determined by the API 20NE MicroTest system, the following characteristics are always negative: reduction of nitrate, production of indole, production of acid from glucose, urease, arginine dihydrolase, and β-galactosidase activities, liquefaction of gelatin, hydrolysis of esculin, and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, maltose, and

D-gluconate. Assimilation of adipate, malate, citrate, and phenyl acetate and oxidase activity are always positive. Assimilation of caprate is strain dependent. The API 20NE profile of most strains is 0000077; the profile of other strains is 0000067, which is identical to the profile of *B. avium* strains.

The major fatty acid components of all of the strains examined are 16:0, 17:0 cyclo, and summed feature 3 (probably 14:0 3OH). Ubiquinone 8 and trace amounts of ubiquinone 9 are the respiratory quinones.

Most strains have been isolated from respiratory tracts of turkeys and chickens. Experimental infections in turkey poults have shown that *B. hinzii* is nonpathogenic (19). Recent studies at the Animal Research Institute in Yeerongpilly, Australia, have shown that the Australian isolates of *B. hinzii* are nonpathogenic for 1-day-old chickens and turkey poults (2). The available clinical histories do not provide strong evidence that this organism plays a role in respiratory disease (3, 18). Two strains have been isolated from humans; one was responsible for bacteremia, and the other was isolated from sputum.

The DNA base composition is between 65 and 67 mol% G+C. The type strain is LMG 13501 (= TC58), which was isolated from a chicken trachea in Australia; its G+C content is 66 mol%.

All *B. hinzii* strains have been deposited in the Laboratorium Microbiologie Gent Culture Collection.

Taxonomic position of strain LMG 13506. Strain LMG 13506 was isolated in a mixed culture from an ear swab of a patient suffering from chronic otitis media (14). This strain exhibited the typical biochemical reactions of *B. avium* but had a different pattern of antimicrobial resistance. DNA-rRNA hybridization data confirmed that this strain belongs to the family *Alcaligenaceae* (Table 4). However, the absence of significant DNA binding values when this organism was tested with reference strains belonging to all *Bordetella* and *Alcaligenes* species that have been described indicates that it represents yet another novel species within this rRNA cluster. We provisionally designated this strain *Bordetella* sp. as it resembles or is virtually indistinguishable from *B. avium* in its biochemical, protein, and fatty acid profiles (Fig. 1 and Table 2) (14).

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