Bordetella trematum sp. nov., Isolated from Wounds and Ear Infections in Humans, and Reassessment of *Alcaligenes denitrificans* Rüger and Tan 1983

P. VANDAMME,^{1*} M. HEYNDRIX,¹ M. VANCANNEYT,¹ B. HOSTE,¹ P. DE VOS,¹
E. FALSEN,² K. KERSTERS,¹ AND K.-H. HINZ³

Laboratorium voor Microbiologie, University of Ghent, Ghent, Belgium¹; Department of Clinical Bacteriology, University of Göteborg, Göteborg, Sweden²; and Klinik für Geflügel der Tierärztlichen Hochschule, Hannover, Germany³

Ten strains recognized on the basis of a computer-assisted numerical comparison of whole-cell protein patterns as members of a novel species belonging to the family *Alcaligenaceae* were examined by using an integrated phenotypic and genotypic approach. This species, for which we propose the name *Bordetella trematum* sp. nov., was more closely related to the type species of the genus *Bordetella* (*Bordetella pertussis*) than to the type species of the genus *Alcaligenes* (*Alcaligenes faecalis*) and had the general characteristics of members of this family (i.e., a DNA base ratio in the range from 57 to 70 mol%, a fatty acid profile characterized by high percentages of 16:0, 17:0 cyclo, and 14:0 3OH, nonsaccharolytic metabolism, and several classical biochemical characteristics, including aerobic and microaerobic growth, catalase activity, assimilation of citrate, an absence of anaerobic growth, and an absence of acetylmethylcarbinol and indole production, gelatin liquefaction, and esculin hydrolysis). A reevaluation of the criteria used to classify *Alcaligenes denitrificans* Rüger and Tan 1983 and *Achromobacter xylosoxidans* Yabuuchi and Ohyama 1971 as subspecies of *Alcaligenes xylosoxidans* and additional evidence provided in recent studies revealed that, consistent with present standards, it is appropriate to consider these two taxa distinct species of the genus *Alcaligenes*.

Members of the genera *Bordetella* and *Alcaligenes* occur in different ecological niches. Some are strict parasites of humans, and others are detected in several hosts, including humans, or are isolated from humans, animals, and the environment (16, 18, 36). *Bordetella pertussis*, the causative agent of whooping cough, and *Bordetella parapertussis* are the most relevant members with respect to human medicine. *Bordetella bronchiseptica* and *Bordetella avium* are respiratory pathogens in various animal hosts; the former occasionally infects humans as well (36). *Bordetella holmesii* and *Bordetella hinzii* are novel species. *B. holmesii* is isolated from human blood samples and has never been associated with the respiratory tract, while *B. hinzii* is a nonpathogenic inhabitant of the respiratory tracts of fowl which occasionally infects humans (3, 10, 31). All *Alcaligenes* species (*Alcaligenes xylosoxidans* subsp. *xylosoxidans*, *Alcaligenes xylosoxidans* subsp. *denitrificans*, *Alcaligenes piechaudii*, and *Alcaligenes faecalis*) occur in human sources other than the respiratory tract and in the environment (16, 18).

Several atypical or unclassified *bordetellae* have been deposited in our laboratory collection for further examination. In the present study, we performed an integrated genotypic and phenotypic analysis of 10 strains which represent a novel species, for which we propose the name *Bordetella trematum*. Using present standards for species delineation, we also evaluated the evidence used to classify *Alcaligenes denitrificans* Rüger and Tan 1983 and *Achromobacter xylosoxidans* Yabuuchi and Ohyama 1971 as subspecies of *Alcaligenes xylosoxidans* (16). We propose that the subspecies classification should be abandoned and that these taxa should be considered distinct species of the genus *Alcaligenes* (i.e., *Alcaligenes xylosoxidans* and *Alcaligenes denitrificans*, respectively).

MATERIALS AND METHODS

Bacterial strains and growth conditions. All of the strains studied and their sources are listed in Table 1. Bacteriological purity was checked by plating and examining living cells by phase-contrast microscopy and Gram-stained cells. Strains were stored as lyophilized cultures.

B. pertussis strains were grown on charcoal agar (Oxoid, Basingstoke, United Kingdom) supplemented with 10% horse blood unless indicated otherwise. All other strains were grown on Trypticase soy agar (BBL, Becton Dickinson Microbiology Systems, Cockeysville, Md.) and were incubated aerobically at 36 to 37°C unless indicated otherwise. Representative strains of *B. parapertussis* and *B. bronchiseptica* were also grown on charcoal-blood agar to evaluate the influence of the growth medium on the whole-cell protein patterns of these bacteria.

PAGE of whole-cell proteins. Cells were incubated for 48 h. Preparation of cellular protein extracts, polyacrylamide gel electrophoresis (PAGE), a densitometric analysis, normalization and interpolation of the protein profiles, and a numerical analysis were performed as described by Pot et al. (26) by using the GelCompar 3.1 software package (Applied Maths, Kortrijk, Belgium). The profiles were recorded and stored on a PC computer. The levels of similarity between pairs of traces were expressed by the Pearson product moment correlation coefficient converted for convenience to a percentage.

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 (32).

Preparation of DNA. Rapid, small-scale DNA extractions for PCR analysis were performed as described by Pitcher et al. (24), with the following slight modifications. Cells grown in petri dishes containing Trypticase soy agar for 24 h at 37°C were washed with saline buffer (0.15 M NaCl, 0.01 M EDTA; pH 8.0), and the purified DNA was treated with RNase at a final concentration of 250 µg/ml of TE buffer (10 mM Tris-HCl, 1 mM EDTA; pH 8.0) for 1 h at 37°C. The quality of the purified DNA was monitored by 1% (wt/vol) agarose gel electrophoresis, and the DNA preparations were quantified by determining the A_{260} .

Large-scale, high-molecular-weight native DNA was prepared as described previously (32).

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 (5).

DNA-DNA hybridization experiments. Degrees of DNA-DNA binding, expressed as percentages, were determined spectrophotometrically by the initial renaturation rate method of De Ley et al. (7). Each value given below is the average of the values from at least two hybridization experiments. When this method is used, DNA binding values of 30% and less do not represent significant levels of DNA homology. 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.

Amplified ribosomal DNA restriction analysis (ARDRA). The 16S rRNA

* Corresponding author. Mailing address: Laboratorium voor Microbiologie, K. L. Ledeganckstraat 35, B-9000 Ghent, Belgium. Phone: (32)9.264.51.13. Fax: (32)9.264.53.46. Electronic mail address: Peter.Vandamme@rug.ac.be.

TABLE 1. Strains studied

Strain ^a	Species	Other designation(s) ^a	Depositor ^{a,b}	Source
LMG 1229 ^T	<i>Alcaligenes faecalis</i>	NCIB 8156 ^T , CCM 1052 ^T	NCIB, CCM	Unknown
LMG 1230	<i>Alcaligenes faecalis</i>	CCEB 554	CCEB	Feces, Czechoslovakia
LMG 3343	<i>Alcaligenes faecalis</i>	NCTC 415	NCTC	Feces
LMG 1861	<i>Alcaligenes piechaudii</i>	NCMB 1051	Torry Research Station	Soil
LMG 1873 ^T	<i>Alcaligenes piechaudii</i>	CIP 60.75 ^T	CIP	Pharynx
LMG 1231 ^T	<i>Alcaligenes denitrificans</i>	ATCC 15173 ^T , NCTC 8582 ^T	ATCC, NCTC	Soil
LMG 1863 ^T	<i>Alcaligenes xylosoxidans</i>	ATCC 27061 ^T	E. Yabuuchi	Human ear discharge, Japan
LMG 1851	<i>Bordetella avium</i>	IPDH 363-78		Turkey with turkey coryza, respiratory tract, Germany
LMG 1852 ^T	<i>Bordetella avium</i>	IPDH 591-77 ^T		Turkey, air sac exudate, Germany
LMG 10978	<i>Bordetella avium</i>	Hommez 20	Hommez	Trachea, broiler with slight tracheitis and polyserositis, Torhout, Belgium, 1988
	<i>Bordetella avium</i>	IPDH 383-78		Turkey, trachea, Germany
LMG 1232 ^T	<i>Bordetella bronchiseptica</i>	NCTC 452 ^T	NCTC	Dog, lung
LMG 14526	<i>Bordetella bronchiseptica</i>	CCUG 31300	CCUG	Human pharynx, Sweden
LMG 3529	<i>Bordetella bronchiseptica</i>	NCTC 454	NCTC	Rabbit, blood
	<i>Bordetella bronchiseptica</i>	IPDH 45-95		Turkey, trachea, Germany
LMG 13501 ^T	<i>Bordetella hinzii</i>	TC58 ^T	Blackall	Chicken trachea, Australia
LMG 14052	<i>Bordetella hinzii</i>		Cookson	AIDS patient, United States
LMG 15872	<i>Bordetella hinzii</i>	DMMZ 1281	Funke	Cystic fibrosis patient, Switzerland
LMG 15047	<i>Bordetella holmesii</i>	CDC G9011	Cookson	Human blood, 80-yr-old female, United States
LMG 15945 ^T	<i>Bordetella holmesii</i>	CCUG 34073 ^T	CCUG	Human blood, United States
LMG 15946	<i>Bordetella holmesii</i>	CCUG 34074	CCUG	Human blood, United States
LMG 1816	<i>Bordetella parapertussis</i>	NCTC 7385	NCTC	Unknown
LMG 14449 ^T	<i>Bordetella parapertussis</i>	CCUG 413 ^T , NCTC 5952 ^T	CCUG, NCTC	Whooping cough
LMG 14522	<i>Bordetella pertussis</i>	CCUG 32523	CCUG, NCTC	Human, Sweden, 1994
LMG 14455 ^T	<i>Bordetella pertussis</i>	CCUG 30873 ^T , NCTC 10739 ^T	CCUG	Unknown
LMG 15140	<i>Bordetella pertussis</i>	CCUG 13475	CCUG	Whooping cough
LMG 15141	<i>Bordetella pertussis</i>	CCUG 15610	CCUG	Whooping cough
LMG 13506 ^T	<i>Bordetella trematum</i>	1779 ^T	Wirsing von Konig	Chronic otitis media, human, Germany
LMG 14446	<i>Bordetella trematum</i>	CCUG 13902	CCUG	Leg wound, 1980
LMG 14447	<i>Bordetella trematum</i>	CCUG 13905	CCUG	Ankle wound, 1980
LMG 14448	<i>Bordetella trematum</i>	CCUG 31299A	CCUG	Unknown
LMG 14523	<i>Bordetella trematum</i>	CCUG 24727	CCUG	Leg wound, United States, 1983
LMG 14991	<i>Bordetella trematum</i>	CCUG 13903A	CCUG	Leg wound, 1980
LMG 14992	<i>Bordetella trematum</i>	CCUG 13903B	CCUG	Leg wound, 1980
LMG 14993	<i>Bordetella trematum</i>	CCUG 13904	CCUG	Arm wound, 1980
LMG 15543	<i>Bordetella trematum</i>	CCUG 14939	CCUG	United States, 1983
LMG 16652	<i>Bordetella trematum</i>	DMMZ 1733	Funke	31-yr-old male with chronic otitis media, Switzerland

^a ATCC, American Type Culture Collection, Rockville, Md.; Blackall, P. Blackall, Queensland Department of Primary Industries, Animal Research Institute, Yeerongpilly, Queensland, Australia; CCEB, Culture Collection of Entomogenous Bacteria, Department of Insect Pathology, Institute of Entomology, Prague, Czech Republic; CCUG, Culture Collection, University of Göteborg, Department of Clinical Bacteriology, University of Göteborg, Göteborg, Sweden; CDC, Centers for Disease Control and Prevention, Atlanta, Ga.; CIP, Collection bactérienne de l'Institut Pasteur, Paris, France; DMMZ, Department of Medical Microbiology, University of Zürich, Zürich, Switzerland; Funke, G. Funke, Department of Medical Microbiology, University of Zürich, Zürich, Switzerland; Hommez, J. Hommez, Regional Veterinary Investigation Centre, Torhout, Belgium; IPDH, Institute of Poultry Disease Hannover, Hannover, Germany; LMG, Laboratorium Microbiologie Gent Culture Collection, Universiteit Gent, Ghent, Belgium; NCIB, National Collection of Industrial Bacteria, NCIMB, Ltd., Aberdeen, Scotland, United Kingdom; NCMB, National Collection of Marine Bacteria, NCIMB, Ltd., Aberdeen, Scotland, United Kingdom; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, United Kingdom; Wirsing von Konig, C. H. Wirsing von Konig, Institute for Hygiene and Laboratory Medicine, Städtische Krankenhäuser Krefel, Krefel, Germany.

^b Our isolate unless indicated otherwise.

genes were amplified by PCR in 50 µl of PCR buffer (10 mM Tris-HCl [pH 8.4], 1.5 mM MgCl₂, 50 mM KCl) containing 10 nmol of each deoxynucleoside triphosphate (Pharmacia Biotech Benelux, Roosendaal, The Netherlands), 20 pmol of the forward primer (5'-AGTTTGATCTGGCTCAG-3'; positions 10 to 27 [*Escherichia coli* numbering]; Pharmacia), 20 pmol of the reverse primer (5'-TACCTTGTTACGACTTACCCCA-3'; positions 1507 to 1485; Pharmacia), 1.25 U of *EuroTaq* DNA polymerase (Eurogentec, Seraing, Belgium), and 250 ng of template DNA. A Perkin-Elmer model 9600 thermocycler was used with the following program: initial denaturation at 95°C for 7 min, followed by 40 cycles consisting of denaturation at 95°C for 45 s, annealing at 57°C for 30 s, and extension at 72°C for 1 min, and a final extension step at 72°C for 10 min. The presence and yield of the PCR product (length, about 1,500 bp) were monitored by 1% (wt/vol) agarose gel electrophoresis. Restriction digestion of the amplified 16S ribosomal DNA was carried out for 2 h at the incubation temperature recommended by the manufacturer in 20-µl reaction mixtures containing 10 µl of

the PCR product solution, 2 µl of commercially supplied incubation buffer, and 5 U of one of the following restriction enzymes: *Hae*III (Boehringer, Mannheim, Germany), *Hha*I (Pharmacia), *Aha*I (Pharmacia), *Taq*I, or *Bst*UI (New England Biolabs). After 4 µl of sample buffer (4 g of sucrose and 2.5 mg of bromophenol blue in 10 ml of TE buffer) was added, 20 µl of the restriction digest was analyzed by 2% (wt/vol) Metaphor agarose (FMC BioProducts, Rockland, Maine) gel electrophoresis at 3 V/cm for 130 min in 89 mM Tris-89 mM boric acid-2 mM EDTA (TBE buffer) (pH 8.0) containing 0.5 µg of ethidium bromide per ml. After an additional 20 min of staining with 1 µg of ethidium bromide per ml in TBE buffer and 5 min of destaining with 10 mM MgCl₂, the gels were scanned under UV illumination by using the Foto/Analyst Visionary digital documentation system (Fotodyne, Hartland, Wis.) and were digitized with an IRIS video digitizer card and Colorvision software (Inside Technology, Amersfoort, The Netherlands). Normalization of the restriction patterns was performed with the GelCompar version 3.1 software package (Applied Maths) by using plasmid

pBR322 digested with *AluI* (Pharmacia) as the reference. The normalized restriction patterns obtained with the five restriction enzymes for each strain were assembled into a combined profile and were analyzed by using the Dice similarity coefficient and the unweighted pair group with mathematical average clustering algorithm.

Phenotypic tests. Strains were grown on Columbia agar base (Oxoid) supplemented with 7% defibrinated sheep blood unless stated otherwise. Cells used as inocula for biochemical tests were grown for 16 to 24 h under aerobic or microaerobic conditions in a moist chamber. The bacteria were suspended in a 0.15 M NaCl solution (pH 7.0 ± 1) to a concentration of about 10^9 viable bacterial cells per ml unless indicated otherwise.

Growth was evaluated on MacConkey agar (Merck, Darmstadt, Germany), Simmons citrate agar (Oxoid), and tyrosine agar prepared as described by Lautrop (22) by using nutrient agar (Oxoid) supplemented with 0.1% DL-tyrosine (Riedel de Haen, Seelze, Germany). Each Simmons citrate agar slant was inoculated with a loopful of a dense bacterial suspension. Growth on tellurite-containing agar was examined by using MacConkey agar supplemented with 320 mg of potassium tellurite hydrate (Fluka Chemie, Buchs, Switzerland) per liter. In addition, agar plates containing 100 or 50 mg of tellurite per liter were also used.

Capsules and flagella were stained as described by Möller (23) and Walc-Pokrzywnicki (34), respectively, by using of bacterial cells harvested from blood agar cultures after 16 h of incubation at 37°C. Motility was evaluated by microscopic examination of cells grown at 25, 30, or 37°C.

Oxidase activity was tested with a freshly prepared 1% solution of *N,N'*-dimethyl-*p*-phenylene monochloride (Sigma Chemical Co., St. Louis, Mo.) and with the less sensitive reagent of Gaby and Hadley (11). The oxidase activity was determined on reagent-impregnated filter paper as described by Kersters and De Ley (15). The reactions were read within 30 s for the Kovács reagent and within 2 min for the reagent of Gaby and Hadley. Catalase activity was tested as described by Kersters et al. (17). Urea hydrolysis was determined by using Christensen urea agar (Merck), and the tests were read daily for up to 5 days. Nitrate reduction was studied in nitrate broth cultures (Merck), and indole production was determined in standard II nutrient broth (Merck) after 24 and 48 h of incubation at 37°C. Alkalinization of litmus milk was tested in steamed milk containing 7% Kubel-Tieman litmus solution (4). To examine production of acetylmethylcarbinol (Voges-Proskauer test), glucose-phosphate medium containing 0.5% peptone (peptone no. 100; Gibco BRL, Gaithersburg, Md.), 0.5% glucose, and 0.5% K_2HPO_4 (pH 6.9) was used. The reactions were read after 2 and 4 days of incubation by adding the reagent of Barrit (4). To study alkalinization of amides and organic salts, modified Greenwood low-peptone medium was used as described previously (31). Tetrazolium reduction was tested on D. S. T. agar (Oxoid) supplemented with 75 mg of tetrazolium (2,2,5-triphenyltetrazolium chloride; Sigma) per liter.

The API 20NE, API 50 CH, API ZYM, API ID32 E, and rapid ID32 A microtest systems were used as recommended by the manufacturer (bioMérieux, La Balme-les-Grottes, Montalieu-Vercieu, France). All tests were repeated at least twice.

RESULTS

PAGE of whole-cell proteins. The strains examined by whole-cell protein analysis methods are listed in Fig. 1. Duplicate protein extracts were prepared to check the reproducibility of the growth conditions and the preparation of the extracts. The levels of similarity between duplicate protein patterns were at least 94% (data not shown). The protein profiles of *B. parapertussis* and *B. bronchiseptica* strains on Trypticase soy agar and charcoal-blood agar were identical (data not shown), which suggested that comparison of the protein patterns of *B. pertussis* strains grown on charcoal-blood agar with the patterns of other species grown on Trypticase soy agar was justified.

The dendrogram obtained after a numerical analysis of the whole-cell protein patterns is shown in Fig. 1. Eight stable clusters and two strains with separate positions were differentiated. Cluster I comprises all of the *B. trematum* strains, which cluster above a similarity level of 86.7%. Clusters II, III, V, VI, VII, and VIII each comprise the reference strains of a single species (*Alcaligenes faecalis*, *B. hinzii*, *B. avium*, *B. holmesii*, *B. pertussis*, and *Alcaligenes piechaudii*, respectively), which cluster above similarity levels of 91.5, 80.7, 89.4, 93.6, 94.5, and 91.7%, respectively. Cluster IV consists of two subclusters; *B. parapertussis* strains cluster above a similarity level of 95.9%, whereas *B. bronchiseptica* strains cluster above a similarity level of 93.4%. The *Alcaligenes xylosoxidans* and *Alcaligenes denitri-*

FIG. 1. Dendrogram derived from unweighed pair group average linkage of correlation coefficients for the protein patterns of all of the strains studied. The Roman numerals are cluster numbers, as discussed in the text.

ficans type strains occupy separate positions on the dendrogram.

Cellular fatty acid analysis. The average fatty acid methyl ester compositions of *B. trematum* and *B. holmesii* strains and *Bordetella* and *Alcaligenes* reference species are shown in Table 2 (data for the reference species were obtained from a previous study [31]). The major fatty acid components of the *B. trematum* and *B. holmesii* strains, as well as the other species, were 16:0, 17:0 cyclo, and summed feature 3. Summed feature 3 comprises two fatty acids which cannot be distinguished by the Microbial Identification System (16:1 iso I and 14:0 3OH). As discussed previously (31), in contrast to 14:0 3OH, branched-chain fatty acids have not been found previously in *Bordetella* or *Alcaligenes* strains, and therefore, the peak designated summed feature 3 probably corresponds to 14:0 3OH.

DNA base compositions. All of the DNA base ratios determined in this study are shown in Table 3. *B. trematum* strains have DNA base ratios between 64 and 65 mol%.

DNA-DNA hybridization experiments. The DNA-DNA hybridization values indicate that the *B. trematum* strains constitute a homogeneous species (the DNA binding values are 81% or more [Table 3]). No significant DNA binding values with reference strains of other *Bordetella* or *Alcaligenes* species were obtained.

ARDRA. In the numerical analysis of the combined ARDRA patterns (Fig. 2), all of the species except *B. pertussis*, *B. parapertussis*, and *B. bronchiseptica* occupied clearly distinct positions. The *B. trematum* strains clustered together at a similarity

TABLE 2. Average fatty acid compositions of the strains studied

Taxon	No. of strains examined	% of ^a :										
		12:0	12:0 2OH	14:0	14:0 2OH	Summed feature 3 ^b	16:0	16:1 <i>cis</i> 9	16:0 2OH	17:0 cyclo	18:0	18:1 ^c
<i>B. trematum</i>	9	Tr	2.7 ± 0.4	1.1 ± 0.3	4.6 ± 0.6	10.0 ± 1.3	37.5 ± 1.4	ND	2.3 ± 0.8	31.6 ± 1.5	4.2 ± 0.3	Tr
<i>B. avium</i> ^c	14	Tr	3.0 ± 0.9	1.8 ± 0.7	3.8 ± 0.9	9.1 ± 2.3	34.7 ± 3.6	Tr	1.5 ± 1.4	35.3 ± 2.9	3.7 ± 1.6	ND
<i>B. hinzii</i> ^c	14	Tr	3.4 ± 0.4	1.3 ± 0.5	4.6 ± 0.5	10.7 ± 0.9	32.4 ± 2.2	1.4 ± 0.5	2.5 ± 0.7	32.4 ± 1.4	4.0 ± 1.9	Tr
<i>B. holmesii</i>	3	Tr	2.9 ± 0.1	Tr	3.4 ± 0.1	7.9 ± 0.3	41.5 ± 0.7	ND	Tr	34.2 ± 0.5	4.9 ± 1.7	ND
<i>Alcaligenes xylosoxidans</i>	Tr	3.9	Tr	1.1	4.8	11.2	35.2	5.8	1.5	28.4	1.5	Tr
LMG 1863 ^{Te}												Tr
<i>B. bronchiseptica</i> ^c	2	Tr	3.5 ± 1.1	6.7 ± 0.4	ND	9.3 ± 2.6	36.3 ± 4.8	1.8 ± 0.3	ND	34.0 ± 1.8	1.8 ± 0.5	Tr
<i>Alcaligenes piechaudii</i> ^c	2	Tr	3.2 ± 0.1	6.1 ± 0.1	ND	9.1 ± 0.5	31.7 ± 1.0	3.1 ± 0.2	1.0 ± 0.2	35.0 ± 0.9	1.3 ± 0.1	3.7 ± 0.5
<i>Alcaligenes denitrificans</i>	1.6	2.9	Tr	6.4	ND	10.2	35.2	4.3	1.0	29.4	1.2	2.4
LMG 1231 ^{Te}												Tr
<i>Alcaligenes faecalis</i> ^c	2	2.1 ± 0.9	3.4 ± 0.6	1.7 ± 0.1	ND	13.6 ± 2.3	30.0 ± 4.0	2.9 ± 0.8	ND	33.8 ± 1.6	Tr	3.9 ± 1.8

^a The fatty acids that were present at levels of less than 1% (average for all taxa) are not included. Therefore, the percentages may not add up to 100%. Tr, trace (less than 1%); ND, not detected.

^b Summed feature 3 is 16:1 *iso* 1 or 14:0 3OH or both.

^c 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).

^d ECL, equivalent chain length. The identity of the fatty acid is not known.

^e Data from reference 31.

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

Strain	G+C content (mol%)	% DNA-DNA binding with:	
		<i>B. trematum</i> LMG 13506 ^T	<i>B. trematum</i> LMG 14446
<i>B. trematum</i> LMG 13506 ^T	65	100	
<i>B. trematum</i> LMG 14446	64	93	100
<i>B. trematum</i> LMG 14993	64	93	81
<i>B. avium</i> LMG 1852 ^T	62	3	
<i>B. bronchiseptica</i> LMG 1232 ^T	69	30	
<i>B. hinzii</i> LMG 13501 ^T	66	15	
<i>B. holmesii</i> LMG 15945 ^T	60		27
<i>Alcaligenes faecalis</i> LMG 1229 ^T	57	9	
<i>Alcaligenes piechaudii</i> LMG 1873 ^T	65	20	
<i>Alcaligenes xylosoxidans</i> LMG 1863 ^T	69	21	
<i>Alcaligenes denitrificans</i> LMG 1231 ^T	67	19	

level of at least 94%. Within this cluster, two subgroups containing two and seven strains were recognized when restriction enzyme *AluI* was used. With this enzyme, the 318-bp fragment present in strains LMG 14993 and LMG 14523 was split into two smaller fragments (168 and 152 bp) in the other seven strains (data not shown). The *B. trematum* cluster was linked at a similarity level of 89% to its nearest neighbors, *B. hinzii* and *B. avium*. Differentiation of these three species was possible mainly with restriction enzyme *AluI*, although there was also a minor difference between the *HaeIII* patterns of *B. trematum* and *B. hinzii* (data not shown). All other species occupied more remote positions on the dendrogram (Fig. 2).

Alcaligenes faecalis occupied an isolated position on the dendrogram; all other species clustered together above a similarity level of 78%. *B. pertussis*, *B. parapertussis*, and *B. bronchiseptica* formed a tight cluster above a similarity level of 94%, and *B. holmesii* was the nearest neighbor of this cluster. The latter organism could be differentiated from the *B. pertussis* group by using restriction enzyme *AluI* (data not shown).

Phenotypic tests. All of the *B. trematum* strains except LMG 16652 and the type strains of all other species were included in the phenotypic analysis. Additional reference strains, including *Alcaligenes faecalis* LMG 3343, *B. avium* IPDH 383-78, and *B. bronchiseptica* LMG 3529 and IPDH 45-95, were used as well. Phenotypic test results are discussed below and are shown in Table 4.

The reproducibility of individual tests and microtest systems was generally excellent. However, it should be emphasized that the size of the inoculum was important for the outcome of several tests, such as alkali production from diverse substrates. Several characteristics were examined in duplicate or triplicate by using classical tests or different microtest systems or both. Production of indole was always negative (as determined by classical test and microtest systems). Urease activity was present in *B. parapertussis* LMG 14449^T (T = type strain) and in *B. bronchiseptica*, findings which were obtained with both the classical test and microtest systems. A total of 22 tests (urease, arginine dihydrolase, alkaline phosphatase, leucine arylamidase, α- and β-galactosidase, α-fucosidase, α- and β-glucosidase, β-glucuronidase, and N-acetyl-β-glucosamin-

FIG. 2. Dendrogram derived from unweighed pair group average linkage of Dice similarity coefficients for the combined ARDRA patterns of all of the strains studied. *Pseudomonas fluorescens* LMG 1799 was used as an outgroup reference organism.

dase activities; fermentation of mannose; reduction and denitrification of nitrate; indole production; and assimilation of glucose, L-arabinose, mannose, mannitol, N-acetylglucosamine, maltose, and gluconate) belonging to API microtest systems were present in multiple galleries. Identical test results were obtained in all analyses performed.

Different test results were obtained with different methods for nitrate reduction and alkalization of malonate, as shown in Table 4. Several strains reproducibly assimilated esculin as determined with the API 50 CH microtest system, but none of the strains hydrolyzed esculin when the API 20NE system was used.

The following characteristics were positive for all of the *Bordetella* and *Alcaligenes* strains examined: aerobic and microaerobic growth at 37°C; aerobic growth at 30°C; assimilation of citrate; and catalase, esterase C4, leucine, alanine, and glycine arylamidase, and acid phosphatase activities.

The following characteristics were negative for all of the strains examined: anaerobic growth at 37°C; production of acetylmethylcarbinol and indole; gelatin liquefaction; esculin hydrolysis; acidification of L-arabinose, mannitol, maltose, adonitol, rhamnose, inositol, sorbitol, cellobiose, saccharose, trehalose, D- and L-arabitol, galacturonate, 5-keto-gluconate, palatinose, and glucose; assimilation of D- and L-arabinose, mannose, mannitol, N-acetylglucosamine, maltose, glycerol, erythritol, ribose, L-xylose, adonitol, β-methyl-D-xyloside, galactose, fructose, sorbose, rhamnose, dulcitol, inositol, sorbitol,

α-methyl-D-mannoside, α-methyl-D-glucoside, amygdalin, arbutin, salicin, cellobiose, lactose, melibiose, saccharose, trehalose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D- and L-fucose, and D- and L-arabitol; and arginine dihydrolase, ornithine decarboxylase, histidine arylamidase, α- and β-galactosidase, α-fucosidase, α- and β-glucosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, α-maltosidase, 5-bromo-indoxyl ester lipase, and α-mannosidase activities.

All of the remaining characteristics are strain or taxon dependent and are shown in Table 4.

DISCUSSION

Taxonomic status of *Alcaligenes xylosoxidans* subsp. *xylosoxidans* and *Alcaligenes xylosoxidans* subsp. *denitrificans*. The taxonomic history of the organisms presently classified as *Alcaligenes xylosoxidans* subsp. *xylosoxidans* and *Alcaligenes xylosoxidans* subsp. *denitrificans* is complex and was reviewed in detail by Kersters and De Ley in 1984 (16). At that time, these taxa were classified as *Achromobacter xylosoxidans* and *Alcaligenes denitrificans*, respectively. DNA-DNA hybridizations between strains of these two species by the S1 endonuclease method revealed DNA-DNA binding values of 30 to 40% (19). Furthermore, the DNAs of strains belonging to the two species had indistinguishable thermal denaturation values with labeled *Alcaligenes denitrificans* reference rRNA (16). According to the received opinion, these taxa were classified in a single species despite phenotypic differences.

At present, a genomic species is generally considered a group of strains that exhibit about 50 to 70% DNA-DNA binding, and the differences in thermal stability between homologous and heterologous duplexes are at most 5 to 7°C (29, 35). It is considered essential that phenotypic consistency supports this genotypically oriented definition (29, 35). The 30 to 40% DNA-DNA binding level found by Kiredjian et al. (19) is clearly below the limit of the current species standard. Phenotypic consistency would be enhanced if the two taxa were given status as different species, as they can be distinguished on the basis of phenotypic and chemotaxonomic characteristics. Finally, in the past few years, it has been demonstrated with several genera that different species may exhibit a very high level of rRNA cistron similarity, and some strains that have identical 16S ribosomal DNA sequences have been shown to belong to different species (28).

In the present study, we demonstrated that strains of *Alcaligenes xylosoxidans* subsp. *xylosoxidans* and *Alcaligenes xylosoxidans* subsp. *denitrificans* can be readily differentiated by their whole-cell protein (Fig. 1) and fatty acid (Table 2) components and by other phenotypic characteristics (Table 4). DNA-DNA hybridization between the type strains of the two taxa in which the initial renaturation rate method was used revealed a level of DNA-DNA binding of only 26% (31). We believe that the previously published data conform to present standards, and the results of our analyses indicate that it is appropriate to classify these taxa as distinct species within the genus *Alcaligenes*. Formal nomenclatural modifications are not required as both *Alcaligenes denitrificans* and *Alcaligenes xylosoxidans* were validly described (18, 27). The latter name, however, should be used only for strains previously classified as *Alcaligenes xylosoxidans* subsp. *xylosoxidans* (18) or *Achromobacter xylosoxidans* (41). The descriptions of the two species are the descriptions given by Kersters and De Ley (16) for the corresponding subspecies.

Protein electrophoretic identification of species of the family *Alcaligenaceae*. Previous studies revealed the efficacy of SDS-

TABLE 4. Phenotypic characteristics of the strains examined

Characteristic	<i>B. trematum</i> (n = 9) ^a	<i>B. hinzii</i> LMG 13501 ^T	<i>B. avium</i> (n = 2)	<i>B. bronchiseptica</i> (n = 3)	<i>B. pertussis</i> LMG 14455 ^T
Aerobic growth at 25°C	+ ^{b,c}	+ ^c	+ ^c	+ ^c	—
Aerobic growth at 42°C	+	+	+ ^c	+ ^c	—
Growth on:					
MacConkey agar	+	+	+	+	—
MacConkey agar containing 320 mg of tellurite per liter	—	—	—	—	ND
Simmons citrate agar	—	+	—	+	—
Motility	+	+	+	+	—
Diffusible brown pigment	—	—	—	—	ND
Pigment produced from tyrosine	—	—	—	—	ND
Oxidase activity					
Kovács reagent	—	+	+	+	+
Gaby-Hadley reagent	—	+	—	+	+ ^c
Nitrate reduction (classical method)					
Formation of nitrite	V (6) ^{c,d}	—	—	V	—
Denitrification	V (1)	—	—	V	—
Nitrate reduction (API 20NE test)					
Formation of nitrite	V (6)	—	—	+	—
Denitrification	—	—	—	—	—
Urease activity	—	—	—	+	—
Lysine decarboxylase activity	—	—	—	—	—
Tetrazolium reduction	+	+	+	+	ND
Alkalinization of litmus milk	— (1) ^c	—	—	+	ND
Alkali production from:					
Acetamide	V (8) ^{c,f}	+	+ ^f	—	ND
Adipate	+	+	+ ^f	+	ND
Glycine	—	+ ^c	—	+	ND
Propionamide	+	+	+ ^c	+	ND
Valerate	V (8) ^c	+	—	+ ^c	ND
Malonamide	+	+	—	+ ^c	ND
Malonate	+	+	—	+ ^c	ND
Malonate (API ID32 E test)	—	—	—	—	—
Glucose fermentation	—	—	—	—	—
Assimilation of:					
D-Glucose	—	—	—	—	—
D-Xylose	—	—	—	—	—
D-Gluconate	—	—	—	—	—
Caprate	—	+ ^c	—	V	—
Adipate	+	+	+ ^c	+ ^c	—
L-Malate	+	+	+ ^c	V	—
Phenylacetate	+	+	+	+	—
Esculin	+	+	—	—	—
5-Keto-gluconate	V (7)	—	—	—	—
Browning on 2-keto-gluconate	—	+	—	—	—
Alkaline phosphatase activity	+ ^c	+	V	V	—
Ester C8 lipase activity	+ ^c	+ ^c	V	V	+
Lipase C14 activity	V (1) ^c	—	—	—	—
Trypsin activity	—	—	—	—	—
Chymotrypsin activity	V (1)	—	V	—	+
Naphthol-AS-B1-phosphohydrolase activity	+ ^c	+ ^c	—	—	+ ^c
Arylamidase activity with:					
Valine	+ ^c	+ ^c	V	—	—
Cystine	—	—	—	—	—
Arginine	V (2)	+	V	+	+
Proline	—	—	—	—	ND
Leucyl glycine	+	+	+	+	+
Phenylalanine	V (5)	—	+	—	+
Pyroglutamic acid	—	—	—	—	—
Tyrosine	V (8)	+	+	+	+
Glutamyl glutamic acid	V (1)	—	—	—	+
Serine	+	+	+	+	+
L-Aspartic acid	+ ^c	+	+ ^c	+	+ ^c

^a n is the number of strains studied.^b +, positive; —, negative; V, reaction is strain dependent; ND, not determined.^c Some strains may exhibit a weak reaction.^d The numbers in parentheses are the numbers of strains that exhibit a positive reaction.^e No growth occurs.^f Strains exhibit a positive reaction only if a heavy inoculum is used.

TABLE 4—Continued

<i>B. parapertussis</i> LMG 14449 ^T	<i>B. holmesii</i> LMG 15945 ^T	<i>Alcaligenes denitrificans</i> LMG 1231 ^T	<i>Alcaligenes xylosoxydans</i> LMG 1863 ^T	<i>Alcaligenes piechaudii</i> LMG 1873 ^T	<i>Alcaligenes faecalis</i> (n = 2)
—	—	+ ^c	+ ^c	+	+ ^c
—	—	—	+ ^c	—	+ ^c
+	—	+	+	+	+
+ ^c	—	+ ^c	—	—	—
—	—	+	+	+	+
—	—	+	+	+	+
+ ^c	+ ^c	—	—	—	—
+	+	—	—	—	—
—	—	+	+	+	+
—	—	+	+	+	+
—	—	+	+	+	—
—	—	+	+	—	—
—	—	+	—	+	—
—	—	—	+	—	—
+	—	—	—	—	—
+	—	+	+	—	—
—	— ^e	+	+ ^e	+	+
—	—	+	+	—	V
—	—	+	+	+	+
—	—	+	+	+	—
—	—	+	+	+	+
—	—	+	+	+	+
—	—	+	+	+	V
—	—	+	+	+	+ ^c
—	—	+	+ ^c	+	+
—	—	—	—	—	+
—	—	—	+ ^c	—	—
—	—	—	+	—	—
—	—	—	+	+	—
—	—	—	+ ^c	+	+
—	—	+ ^c	+	+	+
—	—	+	+	—	+
—	—	—	—	—	—
—	—	—	—	—	V
+	+	—	—	—	V
—	+ ^c	+ ^c	+ ^c	+ ^c	+ ^c
—	—	+	+ ^c	+ ^c	+ ^c
—	—	—	—	—	V
—	+	+	+	+	+
—	+	+	+	+	+
—	+	+	+	—	—
—	—	+	+	+	—
—	—	+	+	+	—
—	—	+	+	+	+
—	—	—	—	—	—
—	—	—	+	+	+
+	—	+	+	+	+ ^c

PAGE of whole-cell proteins and computer-assisted numerical comparisons of the protein electrophoretic traces for delineating species of the family *Alcaligenaceae* (30, 31). Strain LMG 13506^T was previously described as a member of this family,

although it did not belong to one of the established nomenclatures (9, 31). A protein electrophoretic analysis of various atypical *Bordetella*-like strains that were mainly isolated from infected wounds (Table 1) revealed substantial similarity

between nine isolates and strain LMG 13506^T (Fig. 1, cluster I). The numerical analysis revealed that all 10 of these strains had very similar protein patterns, which were distinct from the patterns of other *Bordetella* and *Alcaligenes* species, including *B. holmesii*. DNA-DNA hybridization data confirmed that the cluster I strains formed a genotypically homogeneous species (DNA-DNA binding values, >80%), while the DNA binding values with *B. holmesii* or representative strains of the other members of these genera were low or insignificant (Table 3).

Taxonomic position of *B. trematum*. The taxonomic position of strain LMG 13506^T was determined by DNA-rRNA hybridization experiments, which revealed that this organism belonged to the family *Alcaligenaceae* (31). Strain LMG 13506^T is genotypically, phenotypically, and chemotaxonomically representative of its group and was chosen as the type strain of a new species, for which we propose the name *B. trematum* (see below). Conclusions concerning the phylogenetic affiliation of this strain are therefore valid for the entire species.

The levels of DNA-rRNA hybridization obtained with labeled rRNAs of the type strains of various *Bordetella* and *Alcaligenes* species and the results of an ARDRA in which five different restriction enzymes were used revealed that *B. trematum* was more closely related to the type species of the genus *Bordetella* (*B. pertussis*) than to the type species of the genus *Alcaligenes* (*Alcaligenes faecalis*) (31) (Fig. 2). Thus, this new species genotypically belongs to the genus *Bordetella*. Phenotypically, the genera *Bordetella* and *Alcaligenes* are difficult to differentiate. Hybridization between rRNA and genomic DNA first revealed the close relationship between the two genera (8, 16), and this was later confirmed by the results of a sequence analysis of 16S rRNAs (2, 38). These two genera constitute the family *Alcaligenaceae*, which belongs to the beta subclass of the *Proteobacteria* (6). When the descriptions of the two genera in *Bergey's Manual of Systematic Bacteriology* and the descriptions of the species named after publication of *Bergey's Manual* (18, 31, 38) are compared, it is obvious that phenotypic differentiation at the genus level is virtually impossible. Traditionally, *Bordetella* species are known as respiratory tract parasites, whereas *Alcaligenes* species are considered primarily inhabitants of soil and water (16, 25). However, insights into the phylogeny of these bacteria and the recent emergence of additional species have blurred this picture. The majority of the *Alcaligenes* species have been shown to be only remotely related to *Alcaligenes faecalis*, the type species of the genus (16), and have been gradually reclassified in new genera, such as the genera *Deleya* (1) and *Variovorax* (39). The remaining genuine *Alcaligenes* species, *Alcaligenes faecalis*, *Alcaligenes xylosoxidans*, *Alcaligenes denitrificans*, and *Alcaligenes piechaudii*, all occur in environmental and human sources (16, 18). On the other hand, *Bordetella* species can no longer be considered strict respiratory pathogens. Indeed, *B. trematum* has never been associated with the respiratory systems of humans or animals (38), while the available data indicate that *B. hinzii* is an innocent colonizer of the respiratory tracts of poultry (31). In addition to the human *B. hinzii* isolates described previously (3, 31), three additional isolates were identified recently, including two strains that chronically colonized a cystic fibrosis patient (10) and a strain obtained from an endotracheal aspirate of a farmer (data for the latter strain pointed toward colonization, not infection) (33).

As mentioned previously, *Alcaligenes xylosoxidans*, *Alcaligenes denitrificans*, and *Alcaligenes piechaudii* are genotypically more closely related to the type species of the genus *Bordetella* than to the type species of the genus *Alcaligenes* (2, 8, 38). Their DNA base ratios are also within the range of values obtained for *Bordetella* species (63 to 70 mol%), while the

DNA base ratio of *Alcaligenes faecalis* (55 to 60 mol%) is significantly lower (8). Reclassification of *Alcaligenes xylosoxidans*, *Alcaligenes denitrificans*, and *Alcaligenes piechaudii* in the genus *Bordetella* would enhance the genotypic homogeneity and boundaries of both genera. In the present classification, genus level differentiation by phenotypic characteristics is not possible; however, this would not be achieved by the reclassification of *Alcaligenes* species as suggested above. Such a reclassification would also not serve practical purposes, and therefore we do not propose nomenclatural modifications.

Differentiation of *B. trematum* from allied species. As explained above, we originally recognized strains as members of *B. trematum* on the basis of the results of SDS-PAGE of whole-cell proteins. An extensive phenotypic comparison of *B. trematum* and the type and reference strains of other species revealed several differential characteristics for each of the taxa examined, as shown in Table 4. A number of discrepancies between our results and other data were noted. Our results for tetrazolium reduction and growth on tellurite-containing agar plates for the *B. bronchiseptica* and *Alcaligenes* strains did not correspond to the results of Johnson and Sneath (14). No growth was observed even when the tellurite concentration was reduced to 100 or 50 mg/liter. In light of these results, it seems doubtful that these tests can be used for characterization of these species. Also, *B. holmesii* LMG 15945^T did not grow on MacConkey agar, even after incubation for up to 5 days, while Weyant et al. (38) reported that most *B. holmesii* strains grew after 2 days of incubation and that all strains grew after 7 days of incubation. In contrast to data reported previously (31), in the present study *Alcaligenes faecalis* strains produced alkali from acetamide. However, it should be noted that the results of the alkali production test on Greenwoods low-peptone medium depend on the size of the inoculum used. Likewise, in contrast to the data in Table 4, all of the strains examined alkalized Simmons citrate agar if heavy inocula were used (data not shown).

In the present phenotypic analysis, we included one or a few reference strains for each taxon, yet most of the data shown in Table 4 agree (see above) with previously published data which were obtained with larger numbers of strains (16–18, 25, 31, 38). Among the tests listed in Table 4, both oxidase tests, the urease test, and the test for growth on Simmons citrate agar are particularly useful as first screening tests.

Cellular fatty acid analysis is often used to identify and differentiate bacteria (37). We compared the fatty acid profiles of *B. trematum* and *B. holmesii* strains with the profiles of the other species examined previously (31). From the data in Table 2, we deduced that cellular fatty acid analysis can be used to differentiate some species belonging to the *Bordetella-Alcaligenes* group. The fatty acid 14:0 2OH is useful for separating two main clusters of species, a cluster containing *B. trematum*, *B. avium*, *B. hinzii*, *B. holmesii*, and *Alcaligenes xylosoxidans* on the one hand, and a cluster containing *B. bronchiseptica*, *Alcaligenes piechaudii*, *Alcaligenes denitrificans*, and *Alcaligenes faecalis* on the other hand. Within the first cluster, *Alcaligenes xylosoxidans* can be easily differentiated from the other species by using the ratio of 16:1 *cis* 9 to 17:0 cyclo. Only 16:0 has additional discriminatory power for some species of this first cluster. As demonstrated by Cookson et al. (3) and Funke et al. (10), a shorter incubation time (24 versus 48 h) resulted in a very different ratio of 16:1 *cis* 9 to 17:0 cyclo, which allowed clear differentiation of *B. avium* and *B. hinzii* (16:1 *cis* 9 is a precursor of 17:0 cyclo and therefore is present at higher levels in younger cells). Within the second cluster, *Alcaligenes faecalis* can be differentiated from the other species by its low level of 14:0. The percentage of 18:1 and the ratio of 16:1 *cis* 9 to 17:0

cyclo seem to be useful for differentiating *B. bronchiseptica*, *Alcaligenes piechaudii*, and *Alcaligenes denitrificans*, but a larger number of strains must be examined to conclude that these features have diagnostic value.

ARDRA has been used to differentiate species belonging to different genera (12), including the genera *Capnocytophaga* (40), *Streptococcus* (13), and *Rhizobium* (21). In the present analysis, we included nine *B. trematum* strains and reference strains of all other *Bordetella* and *Alcaligenes* species. The ARDRA patterns obtained with five different restriction enzymes were combined and used in a numerical comparison analysis (Fig. 2). *B. trematum* strains formed a single cluster that was clearly distinct from the other species examined. The subdivision of the *B. trematum* strains into two clusters on the basis of restriction enzyme *AluI* data was, to our knowledge, not supported by other genotypic or phenotypic characteristics, and strains belonging to both subclusters exhibited high levels of DNA-DNA binding with the type strain, LMG 13506 (Table 3). All other species except *B. pertussis*, *B. parapertussis*, and *B. bronchiseptica* are clearly separated on the dendrogram, indicating that this method is very useful for species differentiation in the family *Alcaligenaceae*. In view of the high levels of genotypic relatedness of *B. pertussis*, *B. parapertussis*, and *B. bronchiseptica*, as deduced from DNA-DNA hybridization studies (20, 31), it is not surprising that these species form a single cluster. When the topology of the dendrogram based on five ARDRA patterns is compared with the topology of a dendrogram based on direct 16S rRNA sequencing data, it is noteworthy that both dendrograms indicate that *Alcaligenes faecalis* is the most remote member of the group and that the other *Bordetella* and *Alcaligenes* species are closely intertwined (38) (Fig. 2).

Description of *Bordetella trematum* sp. nov. *Bordetella trematum* (tre.ma'tum. Gr. neut. n. *trema*, referring to something pierced or penetrated, an aperture, or a gap; N. L. gen. pl. n. *trematum*, pertaining to penetrated or open things, referring to the presence of these bacteria in wounds and other exposed parts of the human body). *B. trematum* cells are gram-negative, non-spore-forming, capsulated rods that are motile by means of peritrichous flagella. Motility does not differ significantly when cells are grown at 25, 30, or 37°C. In 16- to 24-h-old cultures on blood agar, the average cell is 0.5 to 0.6 µm wide and 1 to 1.8 µm long; the longest rods are up to 2.4 µm long. Strains produce convex, circular, and greyish cream white colonies with entire edges on blood agar. The strains do not require special growth factors and grow on conventional media. Growth is not inhibited at an incubation temperature of 42°C, but is reduced markedly at 25°C. Strains grow microaerobically, but not anaerobically. Colonies grown for 16 to 24 h on transparent D. S. T. agar at 37°C exhibit greenish yellow to yellow-red iridescence in obliquely transmitted light under a stereomicroscope. Other biochemical characteristics are described above and in Table 4.

The major fatty acid components of all of the strains examined are 12:0 2OH (about 3%), 14:0 2OH (about 5%), 16:0 (about 37%), 17:0 cyclo (about 32%), 16:0 2OH (about 2%), 18:0 (about 4%), and summed feature 3 (probably 14:0 3OH) (about 10%).

Strains have been isolated from ear infections and wounds of humans, but not from respiratory systems. Pathogenic significance is unknown.

The DNA base composition is between 64 and 65 mol% G+C. The type strain is strain LMG 13506, which was isolated from a chronic ear infection in Germany. Its G+C content is 65 mol%.

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

ACKNOWLEDGMENTS

We thank Urbain Torck, Dirk Dewettinck, and Rita Leise for excellent technical assistance and all depositors of strains listed in Table 1.

P.V. and M.H. are indebted to the National Fund for Scientific Research (Belgium) for positions as postdoctoral research fellows. K.K. is indebted to the Fund for Medical Scientific Research, Belgium, for research and personnel grants. Part of this study was performed in the framework of CEC BRIDGE project BIO2-CT94-3098.

REFERENCES

- Baumann, L., R. D. Bowditch, and P. Baumann. 1983. Description of *Deleya* gen. nov. created to accommodate the marine species *Alcaligenes aestus*, *A. pacificus*, *A. cupidus*, *A. venustus*, and *Pseudomonas marina*. Int. J. Syst. Bacteriol. 33:793-802.
- Bleumink-Pluym, N. M. C., L. van Dijk, A. H. M. van Vliet, J. W. B. van der Giessen, and B. A. M. van der Zeijst. 1993. Phylogenetic position of *Taylorella equigenitalis* determined by analysis of amplified 16S ribosomal DNA sequences. Int. J. Syst. Bacteriol. 43:618-621.
- Cookson, B. T., P. Vandamme, L. C. Carlson, A. M. Larson, J. V. L. Sheffield, K. Kersters, and D. H. Spach. 1994. Bacteremia caused by a novel *Bordetella* species, "*B. hinzii*." J. Clin. Microbiol. 32:2569-2571.
- Cowan, S. T. 1974. Cowan and Steel's manual for the identification of medical bacteria, 2nd ed. Cambridge University Press, Cambridge.
- De Ley, J. 1970. Reexamination of the association between melting point, buoyant density, and chemical base composition of deoxyribonucleic acid. J. Bacteriol. 101:738-754.
- De Ley, J. 1992. The *Proteobacteria*: ribosomal RNA cistron similarities and bacterial taxonomy, p. 2111-2140. In A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K.-H. Schleifer (ed.), The prokaryotes, 2nd ed., vol. 2. Springer-Verlag, Berlin.
- De Ley, J., H. Cattoir, and A. Reynaerts. 1970. The quantitative measurement of DNA hybridization from renaturation rates. Eur. J. Biochem. 12: 133-142.
- De Ley, J., P. Segers, K. Kersters, W. Mannheim, and A. Lievens. 1986. Intra- and intergeneric similarities of the *Bordetella* ribosomal ribonucleic acid cistrons: proposal for a new family, *Alcaligenaceae*. Int. J. Syst. Bacteriol. 36:405-414.
- Dorittke, C., P. Vandamme, K. H. Hinz, E. M. Schemken-Birk, and C. H. Wirsing von König. 1995. Isolation of a *Bordetella avium*-like organism from a human specimen. Eur. J. Clin. Microbiol. Infect. Dis. 14:451-454.
- Funke, G., T. Hess, A. von Graevenitz, and P. Vandamme. 1996. Characteristics of *Bordetella hinzii* strains isolated from a cystic fibrosis patient over a 3-year period. J. Clin. Microbiol. 34:966-969.
- Gaby, W. L., and C. Hadley. 1957. Practical laboratory test for the identification of *Pseudomonas aeruginosa*. J. Bacteriol. 74:356-358.
- Gürtler, V., and V. A. Stanisich. 1996. New approaches to typing and identification of bacteria using the 16S-23S rDNA spacer region. Microbiology 142:3-16.
- Jayaro, B. M., J. J. E. Doré, Jr., G. A. Baumbach, K. R. Matthews, and S. P. Oliver. 1991. Differentiation of *Streptococcus uberis* from *Streptococcus parauberis* by polymerase chain reaction and restriction fragment length polymorphism analysis of 16S ribosomal DNA. J. Clin. Microbiol. 29:2774-2778.
- Johnson, R., and P. H. A. Sneath. 1973. Taxonomy of *Bordetella* and related organisms of the families *Achromobacteraceae*, *Brucellaceae*, and *Neisseriaceae*. Int. J. Syst. Bacteriol. 23:381-404.
- Kersters, K., and J. De Ley. 1971. Enzymatic tests with resting cells and cell-free extracts. Methods Microbiol. 5A:33-52.
- Kersters, K., and J. De Ley. 1984. Genus *Alcaligenes* Castellani and Chalmers 1919, 936^{AL}, p. 361-373. In N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
- Kersters, K., K.-H. Hinz, A. Hertle, P. Segers, A. Lievens, O. Siegmann, and J. De Ley. 1984. *Bordetella avium* sp. nov., isolated from the respiratory tracts of turkeys and other birds. Int. J. Syst. Bacteriol. 34:56-70.
- Kiredjian, M., B. Holmes, K. Kersters, I. Guilvout, and J. De Ley. 1986. *Alcaligenes piechaudii*, a new species from human clinical specimens and the environment. Int. J. Syst. Bacteriol. 36:282-287.
- Kiredjian, M., M. Popoff, C. Coyault, M. Lefèvre, and M. Lemelin. 1981. Taxonomie du genre *Alcaligenes*. Ann. Inst. Pasteur Microbiol. 132B:337-374.
- Kloos, W. E., N. Mohapatra, W. Dobrogosz, J. W. Ezzell, and C. R. Manclark. 1981. Deoxyribonucleotide sequence relationships among *Bordetella* species. Int. J. Syst. Bacteriol. 31:173-176.
- Laguette, G., M.-R. Allard, F. Revoy, and N. Amarger. 1994. Rapid identi-

- fication of rhizobia by restriction fragment length polymorphism analysis of PCR-amplified 16S rRNA genes. *Appl. Environ. Microbiol.* **60**:56–63.
22. Lautrop, H. 1960. Laboratory diagnosis of whooping cough or *Bordetella* infections. *Bull. W. H. O.* **23**:15–31.
 23. Möller, O. 1951. A new method for staining bacterial capsules. *Acta Pathol. Microbiol. Scand.* **28**:127–131.
 24. Pitcher, D. G., N. A. Saunders, and R. J. Owen. 1989. Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Lett. Appl. Microbiol.* **8**:151–156.
 25. Pittman, M. 1984. Genus *Bordetella* Moreno-Lopez 1952, 178^{AL}, p. 388–394. In N. R. Krieg and J. G. Holt (ed.), *Bergey's manual of systematic bacteriology*, vol. 1. The Williams & Wilkins Co., Baltimore.
 26. Pot, B., P. Vandamme, and K. Kersters. 1994. Analysis of electrophoretic whole-organism protein fingerprinting, p. 493–521. In M. Goodfellow and A. G. O'Donnell (ed.), *Modern microbial methods. Chemical methods in bacterial systematics*. J. Wiley and Sons, Chichester, United Kingdom.
 27. Rüger, H.-J., and T. L. Tan. 1983. Separation of *Alcaligenes denitrificans* sp. nov., nom. rev. from *Alcaligenes faecalis* on the basis of DNA base composition, DNA homology, and nitrate reduction. *Int. J. Syst. Bacteriol.* **33**:85–89.
 28. Stackebrandt, E., and B. M. Goebel. 1994. Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int. J. Syst. Bacteriol.* **44**:846–849.
 29. Ursing, J. B., R. A. Rossello-Mora, E. Garcia-Valdes, and J. Lalucat. 1995. Taxonomic note: a pragmatic approach to the nomenclature of phenotypically similar genomic groups. *Int. J. Syst. Bacteriol.* **45**:604.
 30. Vancanneyt, M., P. Vandamme, and K. Kersters. 1995. Differentiation of *Bordetella pertussis*, *B. paraptussis*, and *B. bronchiseptica* by whole-cell protein electrophoresis and fatty acid analysis. *Int. J. Syst. Bacteriol.* **45**:843–847.
 31. Vandamme, P., J. Hommez, M. Vancanneyt, M. Monsieurs, B. Hoste, B. Cookson, C. H. Wirsing von König, K. Kersters, and P. Blackall. 1995. *Bordetella hinzii* sp. nov., isolated from poultry and humans. *Int. J. Syst. Bacteriol.* **45**:37–45.
 32. Vandamme, P., M. Vancanneyt, B. Pot, L. Mels, B. Hoste, D. Dewettinck, L. Vlaes, C. Van Den Borre, R. Higgins, J. Hommez, K. Kersters, J.-P. Butzler, and H. Goossens. 1992. Polyphasic taxonomic study of the emended genus *Arcobacter*, with *Arcobacter butzleri* comb. nov. and *Arcobacter skirrowii* sp. nov., an aerotolerant bacterium isolated from veterinary specimens. *Int. J. Syst. Bacteriol.* **42**:344–356.
 33. Verhaegen, J. Personal communication.
 34. Walc-Pokrzywnicki, Z. 1973. Flagella staining. *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. I Orig. Reihe A* **223**:240–243.
 35. Wayne, L. G., D. J. Brenner, R. R. Colwell, P. A. D. Grimont, P. Kandler, M. I. Krichevsky, L. H. Moore, W. E. C. Moore, R. G. E. Murray, E. Stackebrandt, M. P. Starr, and H. G. Trüper. 1987. Report of the Ad Hoc Committee on Reconciliation of Approaches to Bacterial Systematics. *Int. J. Syst. Bacteriol.* **37**:463–464.
 36. Weiss, A. A. 1992. The genus *Bordetella*, p. 2530–2543. In A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K.-H. Schleifer (ed.), *The prokaryotes*, 2nd ed., vol. 2. Springer-Verlag, Berlin.
 37. Welch, D. F. 1991. Applications of cellular fatty acid analysis. *Clin. Microbiol. Rev.* **4**:422–438.
 38. Weyant, R. S., D. G. Hollis, R. E. Weaver, M. F. M. Amin, A. G. Steigerwalt, S. P. O'Connor, A. M. Whitney, M. I. Daneshvar, C. W. Moss, and D. J. Brenner. 1995. *Bordetella holmesii* sp. nov., a new gram-negative species associated with septicemia. *J. Clin. Microbiol.* **33**:1–7.
 39. Willems, A., J. De Ley, M. Gillis, and K. Kersters. 1991. *Comamonadaceae*, a new family encompassing the acidovorans rRNA complex, including *Variovorax paradoxus* gen. nov., comb. nov., for *Alcaligenes paradoxus* (Davis 1969). *Int. J. Syst. Bacteriol.* **41**:445–450.
 40. Wilson, M. J., W. G. Wade, and A. J. Weightman. 1995. Restriction fragment length polymorphism analysis of PCR-amplified 16S ribosomal DNA of human *Capnocytophaga*. *J. Appl. Bacteriol.* **78**:394–401.
 41. Yabuuchi, E., and I. Yano. 1981. *Achromobacter* gen. nov. and *Achromobacter xylosoxidans* (ex Yabuuchi and Ohyama 1971) nom. rev. *Int. J. Syst. Bacteriol.* **31**:477–478.