JOURNAL OF CLINICAL MICROBIOLOGY, May 2005, p. 2516–2519 Vol. 43, No. 5
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.5.2516–2519.2005

New Species of *Bordetella*, *Bordetella ansorpii* sp. nov., Isolated from the Purulent Exudate of an Epidermal Cyst

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Received 2 December 2004/Returned for modification 6 January 2005/Accepted 10 January 2005

A gram-negative bacillus, SMC-8986^T, which was isolated from the purulent exudate of an epidermal cyst but could not be identified by a conventional microbiologic method, was characterized by a variety of phenotypic and genotypic analyses. Sequences of the 16S rRNA gene revealed that this bacterium belongs to the genus *Bordetella* but diverged distinctly from previously described *Bordetella* species. Analyses of cellular fatty acid composition and performance of biochemical tests confirmed that this bacterium is distinct from other *Bordetella* species. Furthermore, the results of comparative sequence analyses of two protein-coding genes (*risA* and *ompA*) also showed that this strain represents a new species within the genus *Bordetella*. Based on the evaluated phenotypic and genotypic characteristics, it is proposed that SMC-8986^T should be classified as a new species, namely *Bordetella ansorpii* sp. nov.

The genus *Bordetella* now consists of eight species, including three classical species, *Bordetella pertussis*, *Bordetella parapertussis*, and *Bordetella bronchiseptica* (4, 13). *B. pertussis* and *B. parapertussis* are strict human pathogens causing the respiratory tract infection called whooping cough (6). Even though *B. bronchiseptica* is a commensal of the respiratory tract in many animals, it also infrequently causes respiratory tract infections in humans (3). *Bordetella hinzii*, mainly a colonizer of the respiratory tract of poultry, has been found in immunocompromised humans (11) and was recently reported as a causative agent of fatal septicemia (7). *Bordetella holmesii* and *Bordetella trematum* exclusively infect humans. *B. holmesii* has been found repeatedly in blood of young adults and often in sputum (10, 14). *B. trematum* causes ear and wound infections (12). *Bordetella avium*, a pathogen of birds, causes coryza or rhinotracheitis in poultry, but it has never been found in humans. Lastly, *Bordetella petrii*, which was identified very recently, is a unique member of the genus *Bordetella* isolated from the environment and capable of anaerobic growth (13).

In this paper, we report a novel *Bordetella* species isolated from the purulent exudate of an epidermal cyst. This bacterium could not be identified by a conventional method. Comparative 16S rRNA sequence analysis showed that it belongs to the genus *Bordetella*, but it does not correspond to any previously characterized species. Thus, we suggest a new species name, *B. ansorpii*, for this microorganism based on phenotypic and genotypic characteristics.

Case report. A 19-year-old female was admitted for anticancer chemotherapy. She had received chemotherapy due to rhabdomyosarcoma of the nasal cavity and right orbit since 1 year prior to admission. On admission, a 2-cm, soft, tender, and erythematous mass was detected on her right posterior neck. Her body temperature was normal. The leukocyte count was 5,310/mm^3 (normal range, 3,200 to 9,000/mm^3) with segmented neutrophils at 72% (normal, 40 to 74%) and lymphocytes at 18% (normal, 20 to 50%); the hemoglobin level was 10.2 g/dl (normal, 11.2 to 14.8 g/dl); the erythrocyte sedimentation rate was 21 mm/h (normal, 0 to 27 mm/h); and the C-reactive protein level was 0.07 mg/dl (0 to 0.3 mg/dl). Amoxicillin-clavulanate was given under the impression of the infected mass. As no improvement was observed despite antibiotic therapy for 3 days, the mass was biopsied and the drainage was examined by microscope and cultured. The pathology revealed an infected epidermal cyst. The purulent exudate of an epidermal cyst was cultured on blood and MacConkey agar plates and incubated at 37°C. Gram staining of the colonies on agar plates demonstrated gram-negative bacilli. However, the isolated bacterium could not be identified by conventional automated methods such as VITEK (bioMérieux, Hazelwood, Mo.) and MicroScan (Dade-Microscan, Sacramento, Calif.). The infected mass had improved with amoxicillin-clavulanate after incision and drainage. She received another cycle of chemotherapy uneventfully.

The strain SMC-8986^T, a gram-negative bacillus, grew on both blood and MacConkey agar at 37°C. The VITEK GNI+ card (gram-negative identification card; bioMérieux, Hazelwood, Mo.) and the API 20NE (bioMérieux, Hazelwood, Mo.) were used for identification according to the recommendations of the manufacturer. SMC-8986^T was positive only for citrate utilization in repeated tests with the VITEK GNI+ card. It was identified as *B. avium* with an accuracy of 82% by repeated tests with API 20NE. Briefly, it was positive for gelatin hydrolysis and adipate, malate, citrate, and phenylacetate assimilation, while negative for oxidase, reduction of nitrates to nitrites, indole production, acidification, arginine dihydrolase, urease, β-glucosidase hydrolysis, β-galactosidase, and assimi-

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TABLE 1. Comparison of phenotypic characteristics of *Bordetella* species, including strain SMC-8986^T

Characteristic	Result for:								
	SMC-8986^T	*B. pertussis*	*B. parapertussis*	*B. bronchiseptica*	*B. avium*	*B. hinzii*	*B. holmesii*	*B. trematum*	*B. petrii*
Growth on:									
Blood agar	+	−	+	+	+	+	+	+	+
MacConkey agar	+	−	±	+	+	+	+	+	+
Oxidase	−	+	−	+	+	+	−	−	+
Nitrate reduction	−	−	−	+	−	−	−	±	−
Urease production	−	−	+	+	−	±	−	−	−
Motility	+	−	−	+	+	+	−	+	−

lation of glucose, arabinose, mannose, mannitol, *N*-acetyl-glucosamine, maltose, gluconate, and caprate (Table 1). The organism was motile on LB swarming agar (0.8% NaCl, 0.4% agar [wt/vol]), which distinguishes it from *B. holmesii* (Table 1).

Analysis of cellular fatty acid (CFA) composition was performed for SMC-8986^T, *B. avium* ATCC 35086^T, and *B. brochiseptica* ATCC 4601^T, using a Hewlett Packard 6890A gas chromatograph and the MIDI aerobe method (Chem Station ver. 4.02) at MicroID (Seoul, Korea). The CFA profiles determined in this study were compared with those of several reports from the literature (7, 12, 14). CFA analysis results are shown in Table 2. The cellular fatty acid profile of strain SMC-8968^T was compared with those of other *Bordetella* species and was found to have a similar predominance of $C_{16:0}$ (32.4%). However, the overall CFA composition of SMC-8986^T, which included $C_{16:1\omega7c}$ (17.6%), $C_{18:1\omega7c}$ (12.9%), and $C_{17:0cyclo}$ (9.6%), did not correspond to any previously described *Bordetella* species (Table 2). The lower composition rate of $C_{17:0cyclo}$ is the significant difference between strain SMC-8986^T and other *Bordetella* species. The G+C content of strain SMC-8986^T, which was determined by thermal denaturation (5), was 63.8 mol%. The G+C contents of other *Bordetella* species ranged from 60 to 69 mol% (11–14).

For genotypic characterization, genomic DNA of SMC 8986^T, *B. avium* ATCC 35086^T, and *B. bronchiseptica* ATCC 4617^T was extracted from bacterial colonies by a simple boiling-lysis method (1). Briefly, colonies were suspended in lysis buffer (100 mM NaCl, 10 mM Tris-HCl, 1 mM EDTA, 1% Triton X-100), and were incubated at 80°C for 10 min. The mixture was then centrifuged for a moment, and the aqueous phase was used as a template for PCR. The 16S rRNA was amplified with universal primers 16S-F3 (5′-CAGGCCTAAC ACATGCAAGT-3′) and 16S-R3 (5′-GGGCGGWGTGTAC AAGGC-3′) (15). Template DNA and 50 pmol of each primer were added to a PCR mixture tube (AccuPower PCR PreMix; Bioneer, Daejon, Korea) (8). The reaction mixture was then subjected to 35 cycles for amplification. Each cycle consisted of 30 s at 95°C for denaturation, 30 s at 58°C, and 1 min at 72°C for extension, followed by final extension at 72°C for 5 min. Amplified PCR product was purified for sequencing using a PCR purification kit (CoreOne, Seoul, Korea). The purified PCR product was sequenced directly using the same primers of PCR amplification and another primer, 16S-F5 (5′-TATTGG GCGTAAAGCGAGCGC-3′), which was designed by us. DNA sequences were determined with an ABI prism Rhodomine terminator cycle sequencing kit (PE Biosystems, Foster City, CA) and an ABI 3710 automated sequence (PE Biosystems, Foster City, CA). The determined 16S rRNA nucleotide sequences of SMC-8986^T, *B. avium* ATCC 35086^T, and *B. brochiseptica* ATCC 4617^T (1,424 bp, 1,422 bp, and 1,422 bp, respectively) were used for phylogenetic comparison.

Table 3 presents sequence dissimilarities among 16S rRNA sequences of *Bordetella* species and strain SMC-8986^T, which were analyzed with the MegAlign program in DNASTAR (DNASTAR, Madison, WI). The 16S rRNA gene sequence of SMC-8986^T was compared to published or determined se-

TABLE 2. Comparison of fatty acid composition of *Bordetella* species

Fatty acid	Proportion (%) of fatty acid in:							
	SCM-8986^T	*B. avium*[a]	*B. bronchiseptica*[a]	*B. hinzii*[b]	*B. parapertussis*[b]	*B. pertussis*[b]	*B. holmesii*[b]	*B. trematum*[b]
$C_{12:0aldehyde}$	1.3	0.47	0.48	0.4				
$C_{12:02OH}$	1.1	2.16	2.08	2.34			2.9	2.7
$C_{14:0}$	2.4	0.99	4.83	0.61	6	5		1.1
$C_{14:02OH}$	4.9	2.86		3.38			3.4	4.6
$C_{16:1iso}$	6.7	7.33	6.49	NA[c]	NA	NA	7.9	10.0
$C_{16:1\omega7c}$	17.6	4.31	11.0	3.80	6	40	NA	NA
$C_{16:0}$	32.4	39.13	42.23	34.53	40	32	41.5	37.5
$C_{17:0cyclo}$	9.6	29.52	26.27	33.24	35		34.2	31.6
$C_{16:03OH}$	1.1	0.35	0.23	0.48	NA	NA	NA	NA
$C_{17:0}$		0.92	0.93	0.84	3			NA
$C_{18:1\omega7c}$	12.9	0.67	1.42	1.65				NA
$C_{18:0}$	1.9	4.39	2.80	7.60	5	8	4.9	4.2
$C_{19:0cyclo\omega8c}$	4.6	0.19	0.19	1.26	NA	NA	NA	NA

[a] Fatty acid profiles of *B. avium* ATCC 35086^T and *B. brochiseptica* ATCC 4617^T, which were determined in this study.
[b] Fatty acid profiles retrieved from previous published literature (4, 12, 14).
[c] NA, not applicable.

TABLE 3. Similarities and dissimilarities among 16S rRNA sequences of *Bordetella* species[a]

Species	% Similarity or dissimilarity to:								
	B. holmesii	*B. pertussis*	*B. bronchiseptica*	*B. parapertussis*	*B. hinzii*	*B. trematum*	*B. avium*	*B. petrii*	SMC-8986^T
B. holmesii		99.8	99.5	99.6	99.0	98.4	98.4	98.1	97.4
B. pertussis	0.2		99.8	99.8	99.2	98.6	98.4	98.4	97.5
B. bronchiseptica	0.5	0.2		99.9	99.3	98.6	98.6	98.4	97.6
B. parapertussis	0.4	0.2	0.1		99.5	98.7	98.7	98.4	97.6
B. hinzii	1.0	0.8	0.7	0.5		99.1	99.3	98.2	97.7
B. trematum	1.6	1.4	1.4	1.3	0.9		99.0	98.1	97.7
B. avium	1.6	1.6	1.4	1.3	0.7	1.0		98.0	97.8
B. petrii	1.9	1.6	1.6	1.6	1.8	1.9	2.0		98.3
SMC-8986^T	2.6	2.5	2.4	2.4	2.3	2.3	2.2	1.7	

[a] The strain number of each species is represented in Fig. 1.

quences of other *Bordetella* species, and dissimilarities ranged from 1.7% to 2.6%. Considering the range of dissimilarities (0.1% to 1.9%) among the characterized *Bordetella* species, except SMC-8986^T, and the common difference limit in species definition (1.0%) (2), SMC-8986^T is regarded as a new *Bordetella* species. A phylogenetic tree reconstructed by the method of neighbor joining (9) also suggests a phylogenetic relationship indicating that SMC-8986^T is a member of the genus *Bordetella* but is distinct from other *Bordetella* species (Fig. 1). With respect to the phylogenetic relationships, strain SMC-8986^T was closely related to *B. petrii*, which was supported robustly by the bootstrap value (98%).

In addition to 16S rRNA, the homologues of *risA* and *ompA* genes of *B. avium* were partially amplified and sequenced using the same primers as von Wintzingerode et al. (13). Determined *risA* and *ompA* sequences of SMC-8986^T, *B. avium* ATCC 35086^T, and *B. brochiseptica* ATCC 4617^T were aligned with those of other *Bordetella* species retrieved from GenBank, respectively. *risA* and *ompA* sequences of SMC-8986^T showed 11.2 to 11.6% and 17.2 to 26.3% divergences from those of *B. pertussis*, *B. parapertussis*, *B. bronchiseptica*, *B. avium*, and *B. petrii*, respectively. These results also confirm that strain SMC 8986^T is a distinct species from other *Bordetella* species, although a few *Bordetella* species could not be included in this analysis.

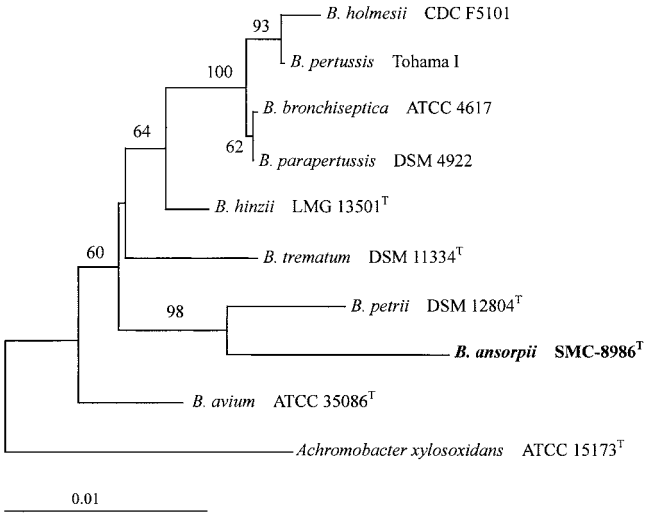

FIG. 1. Phylogenetic relationships of *B. ansorpii* sp. nov. and other *Bordetella* species inferred from 16S rRNA sequences, which were aligned using the multiple alignment program Clustal X. This tree was reconstructed by the neighbor-joining method. *Achromobacter xylosoxidans* ATCC 15173^T was used as an outgroup. Numbers at branching nodes are percentages of 1,000 bootstrap replications. Only values greater than 50% are indicated. The scale bar represents one substitution per 100 nucleotides.

Thus, in light of the complementary nature of all the results described above, in terms of biochemical tests, cellular fatty acid composition, and molecular genetic analysis, a new species of the genus *Bordetella*, *B. ansorpii*, is proposed for strain SMC-8986^T isolated from the purulent exudate of an epidermal cyst.

Description of *Bordetella ansorpii* sp. nov. The species name, *ansorpii*, stands for ANSORP, Asian Network for Surveillance of Resistant Pathogens.

It is a gram-negative bacillus. It grows on both blood agar and MacConkey agar. It is negative for indole production, oxidase, urease, arginine dihydrolase, esculinase, gelatinase, β-galactosidase, nitrate reduction, and assimilation of glucose, mannose, mannitol, *N*-acetyl-glucosamine, malonate, gluconate, and caprate but positive for citrate, adipate, malate, and phenyacetate utilization, gelatinase activity, and motility. It has the cellular fatty acids 16:0, 16:1ω7c, 18:1ω7c, and 17:0cyclo as the major fatty acid components. It was isolated from the purulent exudate of an epidermal cyst, but its pathogenic significance remains unknown so far. It has a G+C content of 63.8 mol%. The type strain of *B. ansorpii* is strain SMC-8986^T, which has been deposited at ABB (Asian Bacterial Bank, Seoul, Korea).

Nucleotide sequence accession number. The sequences of 16S rRNA, *risA*, and *ompA* of strain SMC-8986^T have been deposited in the GenBank database under accession numbers AY594190 to AY594192.

We thank Mi Young Lee and Na Young Kim for technical assistance.

This study was partly supported by the SBRI (Samsung Biomedical Research Institute) (grant C-A4-316-1).

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