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Clinical and Laboratory Features of *Mycobacterium porcinum*†

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Recent molecular studies have shown *Mycobacterium porcinum*, recovered from cases of lymphadenitis in swine, to have complete 16S rDNA sequence identity and >70% DNA-DNA homology with human isolates within the *M. fortuitum* third biovariant complex. We identified 67 clinical and two environmental isolates of the *M. fortuitum* third biovariant sorbitol-negative group, of which 48 (70%) had the same PCR restriction enzyme analysis (PRA) profile as the *hsp65* gene of *M. porcinum* (ATCC 33776^T) and were studied in more detail. Most U.S. patient isolates were from Texas (44%), Florida (19%), or other southern coastal states (15%). Clinical infections included wound infections (62%), central catheter infections and/or bacteremia (16%), and possible pneumonitis (18%). Sequencing of the 16S rRNA gene (1,463 bp) showed 100% identity with *M. porcinum* ATCC 33776^T. Sequencing of 441 bp of the *hsp65* gene showed four sequevars that differed by 2 to 3 bp from the porcine strains. Clinical isolates were positive for arylsulfatase activity at 3 days, nitrate, iron uptake, D-mannitol, i-*myo*-inositol, and catalase at 68°C. They were negative for L-rhamnose and D-glucitol (sorbitol). Clinical isolates were susceptible to ciprofloxacin, sulfamethoxazole, and linezolid and susceptible or intermediate to cefoxitin, clarithromycin, imipenem, and amikacin. *M. porcinum* ATCC 33776^T gave similar results except for being nitrate negative. These studies showed almost complete phenotypic and molecular identity between clinical isolates of the *M. fortuitum* third biovariant D-sorbitol-negative group and porcine strains of *M. porcinum* and confirmed that they belong to the same species. Identification of *M. porcinum* presently requires *hsp65* gene PRA or 16S rRNA or *hsp65* gene sequencing.

The rapidly growing mycobacterial species *Mycobacterium porcinum* was described in 1983 by Tsukamura et al. as a causative agent of submandibular lymphadenitis in swine (23). Tsukamura et al. characterized these strains as being similar to *M. fortuitum* but differing from this species by being D-mannitol and i-*myo*-inositol positive, nitrate negative, and succinamidase positive and utilizing benzoate as a sole source of carbon in the presence of ammoniacal nitrogen. Until recently, no isolation of this species had been reported since that report.

Bönicke studied isolates previously identified as *M. fortuitum* and placed them into three subgroups (1). Subgroup A was negative for D-mannitol and i-*myo*-inositol and now is recognized as *M. fortuitum*. Subgroup B was positive for D-mannitol but negative for i-*myo*-inositol and is now recognized as *M. peregrinum*. Subgroup C was positive for mannitol and inositol, and its taxonomic status has taken much longer to establish and is still evolving. A study by Wallace et al. in 1991 showed this subgroup to consist of two major groups. One group was D-sorbitol positive, had low semiquantitative catalase, and was pipemidic acid resistant, and MICs of cefoxitin (27) and clarithromycin (4) were high for members of the group. β-Lactamase patterns on polyacrylamide gels following isoelectric focusing (IEF) had previously showed these isolates to have primarily a single enzyme pattern (28). These isolates were named the *M. fortuitum* third biovariant complex D-sorbitol-positive group, and ATCC 49403 was chosen as the reference strain (27).

Subsequent 16S rRNA gene sequencing showed these isolates to have a unique hypervariable A region (11, 19) and an 880-bp sequence of ATCC 49403 to differ by 6 to 15 bp from previously recognized species within the *M. fortuitum* group (11). To date, three species are recognized within this D-sorbitol-positive group based on 16S rDNA complete sequencing and DNA-DNA homology studies: *M. mageritense* (7, 26), *M. houstonense* (of which ATCC 49403 is the proposed type strain), and *M. brisbanense* (16).

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† This paper is dedicated to the three coauthors Vella Silcox, Zeta Blacklock, and Michio Tsukamura, early investigators who contributed greatly to the present study but who retired before the present taxonomic status of isolates of the third biovariant complex was established. Both Zeta Blacklock and Michio Tsukamura passed away prior to submission of this study.

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The other major subgroup identified by Wallace et al. (27) within Bonicke's subgroup C (1) was D-sorbitol negative, had a high (height of column of bubbles, >100 mm) semiquantitative catalase, was pipemidic acid susceptible, and was susceptible or intermediate to cefoxitin and clarithromycin (4). This group was named the *M. fortuitum* third biovariant complex D-sorbitol-negative group (27). Subsequent 16S rRNA gene sequencing showed all studied isolates to have an identical but unique hypervariable A region (19). However, IEF studies of the β-lactamase from this group showed a surprising multitude of enzyme patterns, suggesting that multiple taxa or species might be present (32). The first species within this group to be identified was *M. septicum* (17). Only one strain of *M. septicum* has been reported (17), and the majority of isolates of this group were still to be identified to the species level.

This quickly changed when recent molecular studies showed complete sequence identity between the 16S rRNA gene of *M. porcinum* and some of the unnamed isolates of the *M. fortuitum* third biovariant D-sorbitol-negative group (16, 25). Schinsky et al. (16) found four human strains of the third biovariant D-sorbitol-negative group to have 100% sequence identity over 1,401 bp with the *M. porcinum* type strain, ATCC 33776. This latter strain had 91% homology with DNA-DNA comparison to ATCC BAA-328, which is a typical clinical strain of the third biovariant D-sorbitol-negative group.

The original description of *M. porcinum* in 1983 by Tsukamura et al. (23) appears very similar to that of isolates within Bonicke's subgroup C (1) that presently contain the *M. fortuitum* third biovariant, but phenotypic and molecular comparison of these two groups was not done when *M. porcinum* was first described (23). Some phenotypic and drug susceptibility studies on 13 clinical strains of *M. porcinum* were recently reported (16). We compared the type strain of *M. porcinum* and select clinical isolates of the third biovariant D-sorbitol-negative group, including the 13 clinical isolates in the recent taxonomy study (16), by a number of phenotypic and molecular methods to provide additional comparative data for these two taxa. (This work was presented in part as an abstract to the American Society for Microbiology meeting in Washington, D.C., in May 2003.)

MATERIALS AND METHODS

Mycobacterial isolates. Clinical isolates of the *M. fortuitum* third biovariant sorbitol-negative group submitted to the Mycobacteria/Nocardia Laboratory at The University of Texas Health Center at Tyler for identification or susceptibility testing and the type strain of *M. porcinum* (ATCC 33776) were subjected to PCR restriction enzyme analysis (PRA) of the Telenti sequence of the *hsp65* gene (20, 22). Two clinical isolates had previously been submitted to the American Type Culture Collection (ATCC; Manassas, Va.) and numbered as ATCC 49939 and ATCC BAA-328. Information on clinical histories, specimen type, and geographic location was obtained at the time of isolate submission. Chart reviews and informed consent were not obtained. Most clinical information was obtained before current Health Information Patient Protection Agency guidelines were in place. Care was taken not to utilize any specific patient identifiers and to protect patient confidentiality.

Sixty-seven clinical and two environmental isolates of the *M. fortuitum* third biovariant D-sorbitol-negative group were subjected to PRA. Of these, 46 clinical and two environmental isolates (48 isolates or 70%) had a PRA pattern identical to that of *M. porcinum* ATCC 33776^T and were studied for clinical site and geographical source. Among the 45 patient isolates for which culture sites were known, 8 (18%) were respiratory; 7 (16%) were from central catheter-related infections and/or bacteremia and included five known cases of bacteremia; 28 (62%) were from postsurgical (7 cases), posttraumatic (12 cases), or unknown types (9 cases) of wound infections; 1 (2%) was from an infected lymph node; and 1 (2%) was from urine. Seven cases involved known associated osteomyelitis (Table 1). Two environmental isolates were recovered from tap water as part of an investigation of catheter-related infections in a bone marrow transplant unit in Texas (8). When treatment of the patients' infections was based on susceptibility results, with appropriate surgical intervention, all 12 cases with detailed follow-up resulted in clinical resolution.

The 46 isolates for which geographical information was provided were from 16 states and Queensland, Australia. Multiple isolates were obtained from Texas (19 isolates or 41%), Florida (9 isolates or 20%), North Carolina (3 isolates), and Australia (2 isolates). All but eight isolates from the United States (36 of 44 isolates or 82%) were from southern coastal states.

The ATCC reference strains included *M. porcinum* ATCC 33776^T and ATCC 33775, *M. peregrinum* type I ATCC 14467^T, *M. senegalense* ATCC 35796^T and ATCC 13781, *M. neworleansense* ATCC 49404^T, *M. houstonense* ATCC 49403^T, and *M. septicum* ATCC 700731^T.

Growth and biochemical characteristics. Isolates were examined for colony morphology on Mueller-Hinton agar, pigmentation after 1 and 2 weeks (early and late), growth within 7 days on Trypticase soy agar and Middlebrook 7H11 agar at 30°C, and carbohydrate utilization of D-mannitol, i-*myo*-inositol, and D-glucitol (sorbitol) (2, 10, 18, 24, 26, 31). Selected isolates were tested for growth at 35 and 45°C; arylsulfatase activity at 3 days; catalase activity at 68°C; semiquantitative catalase activity; nitrate reductase activity; iron uptake; growth on MacConkey agar; utilization of L-rhamnose, D-trehalose, citrate, benzoate, and acetamide as sole carbon sources (2, 10, 18, 24, 26, 31); and IEF patterns of β-lactamase by using polyacrylamide gels (32). Additionally, most isolates were tested for inhibition by commercial disks of polymyxin B, cephalothin, and kanamycin using agar disk diffusion on Mueller-Hinton agar (Table 2) (26). Results for some isolates have previously been published as part of earlier taxonomic studies (27, 32).

HPLC. Mycolic acids were prepared, esterified, and then subjected to fluorescence detection high-performance liquid chromatography (FL-HPLC) as previously described (2, 26, 31). HPLC reference strains included *M. fortuitum* ATCC 6841^T, *M. peregrinum* ATCC 14467^T, *M. intracellulare* ATCC 13950^T, and *M. neworleansense* ATCC 49404^T.

Susceptibility testing. Susceptibility testing for 11 antimicrobial agents was performed using the broth microdilution method (3, 21) and the recently approved NCCLS standard M24-A (13). Drugs tested were amikacin, tobramycin, cefoxitin, imipenem, doxycycline, ciprofloxacin, gatifloxacin, levofloxacin, clarithromycin, linezolid, and sulfamethoxazole. Control strains included *Staphylococcus aureus* ATCC 29213 and *M. peregrinum* ATCC 700686 (13) (Table 3). Thirty-nine of the 48 isolates (including the two ATCC reference strains) recovered from all time periods were tested against eight of the drugs (the remaining nine isolates had been submitted only for identification, and drug susceptibilities had not been determined), while a smaller number of only recent isolates were tested against three drugs only recently made available (levofloxacin, gatifloxacin, and linezolid).

PRA. Genomic DNA from all isolates was subjected to PCR amplification of the 441-bp Telenti sequence of the *hsp65* gene (20, 22). The PCR product was digested with BstEII and HaeIII, and the DNA restriction fragments were separated using 3% metaphor agarose gels. Selected strains were also cut with BsaHI. Fragment sizes (in base pairs) were estimated using a computerized Bio Image System (Millipore, Bedford, Mass.).

16S rRNA gene sequencing. Selected isolates of the third biovariant complex sorbitol-negative group and the *M. porcinum* type strain underwent sequencing of the first 500 bp using the MicroSeq 500 16S rDNA Bacterial Sequencing Kit (Applied Biosystems, Foster City, Calif.) at Mayo Clinic (9, 14). More complete 16S rRNA gene sequencing was also performed on select isolates using an in-house sequencing protocol (25). This included bases 27 to 1490 (total, 1,463 bases) spanning both hypervariable regions A and B.

***hsp65* partial gene sequencing.** Selected isolates of the third biovariant complex sorbitol-negative group, *M. porcinum* ATCC 33776^T, and *M. porcinum* ATCC 33775 were chosen for sequencing of the 441-bp Telenti fragment of the *hsp65* gene (25). Sequencing of the PCR product obtained using the previously described protocol (22) was performed using the same PCR primers (TB11 and TB12) in a forward and reverse sequencing reaction according to the manufacturer's instructions (Applied Biosystems). Sequence editing and phylogenetic analyses by ClustalV (neighbor-joining method) were performed using the Seqman and Megalign components of Lasergene 5 (DNASTAR).

Nucleotide sequence accession numbers. Examples of the five sequevars of *hsp65* seen with *M. porcinum* have been deposited in GenBank as sequevar 1, accession number AY496137 (ATCC 33776^T); sequevar 2, accession number AY496138 (ATCC BAA-328); sequevar 3, accession number AY496139 (*Mf-*

TABLE 1. Clinical and geographic locations of the 46 clinical and 2 reference isolates of the selected PRA group of the sorbitol-negative third biovariant complex

Isolate type and name	Source	Geographic location	Clinical disease
Respiratory			
Mf-91	Sputum	Florida	Unknown
Mf-131	Lung biopsy	Georgia	Pneumonia
Mf-239	Sputum	Texas	Unknown
Mf-487	Sputum	Texas	Pneumonia
Mf-607	Sputum	Australia	Unknown
Mf-2075	Sputum	Virginia	Unknown
Mf-2084	Sputum	Texas	Bronchiectasis
Mf-2108	Sputum	Illinois	Cystic bronchiectasis
Catheter or blood related			
Mf-115	Blood	Florida	Catheter sepsis
Mf-533	Abdominal wall drainage	Texas	Peritoneal catheter exit site infection
Mf-537	Blood	Texas	Catheter sepsis
Mf-1581	Blood	Texas	Catheter sepsis
Mf-1600	Not specified	Iowa	Catheter sepsis
Mf-1847	Catheter site	Washington	Infected catheter site
Mf-2190	Blood	North Carolina	Hemodialysis related
Wound related			
Mf-114	Leg amputation wound	Texas	Surgical wound infection
Mf-147	Open ankle fracture	Texas	Cellulitis, osteomyelitis, inguinal lymphadenitis
Mf-182	Traumatic knee wound	Texas	Septic arthritis
Mf-205	Surgical abdominal wound	Texas	Surgical wound infection, abdominal wall abscess
Mf-388	Traumatic foot wound	Texas	Cellulitis
Mf-443	Surgical chest wound	Texas	Surgical wound infection
Mf-450	Traumatic leg lesion	Florida	Cellulitis
Mf-485	Open ankle fracture	South Carolina	Cellulitis, osteomyelitis
Mf-611	Sinus, left leg	Australia	Cellulitis
ATCC 49939 (*Mf*-661)	Surgical back wound	Louisiana	Surgical wound infection
Mf-809	Traumatic thigh wound	Texas	Cellulitis
Mf-1363	Abdominal wound	Missouri	Unknown
Mf-1411	Leg wound	Florida	Unknown
Mf-1472	Spinal cord stimulator pocket	Florida	Unknown
Mf-1476	Arm bone tissue	Oklahoma	Osteomyelitis, wound infection
Mf-1533	Leg tissue	Arkansas	Unknown
Mf-1561	Traumatic arm wound	Florida	Osteomyelitis, cellulitis
Mf-1568	Base of toe	Florida	Unknown
Mf-1691	Breast	Texas	Infected implant
Mf-1719	Breast needle aspiration	Alabama	Cellulitis
Mf-1746	Traumatic foot wound	Florida	Cellulitis
Mf-1824	Hand tissue	Florida	Cystic lesion
Mf-1965	Surgical face wound	Texas	Surgical wound infection
Mf-1966	Open ankle fracture	North Carolina	Cellulitis, osteomyelitis
Mf-1997	Traumatic foot wound	Texas	Osteomyelitis, cellulitis
Mf-2083	Breast tissue	Texas	Unknown
Mf-2106	Abscess, unknown site	Unknown	Unknown
Mf-2121	Arm bone tissue	North Carolina	Osteomyelitis, cellulitis
Lymph node related			
ATCC BAA-328 (*Mf*-771)	Inguinal node	Texas	Lymphadenitis
Environmental			
Mf-1615	Water softener	Texas	None
Mf-1616	Water softener	Texas	None
Other			
Mf-2100	Urine	Texas	Unknown
Unknown			
Mf-2107	Unknown	Unknown	Unknown

TABLE 2. Laboratory features of clinical and reference isolates of the selected PRA group of the *M. fortuitum* D-sorbitol-negative third biovariant complex and the ATCC type strain of *M. porcinum*

Isolate group and feature	*M. porcinum* ATCC 33776^T	Third biovariant complex isolates		
		ATCC BAA-328	ATCC 49939	Clinical isolates (no. positive/no. tested) (%)
All isolates				
Pigment (early/late)	−/−	−/−	−/−	0/46 (0)
Rough colony morphology	+	+	+	36/46 (78)
Growth at:				
<7 days	+	+	+	46/46 (100)
30°C	+	+	+	46/46 (100)
45°C[a] (14 days)	+	+	+	14/46 (30)
Acetamide	+	+	+	46/46 (100)
Carbohydrate utilization				
D-Mannitol	+	+	+	46/46 (100)
i-*myo*-Inositol	+	+	+	46/46 (100)
D-Glucitol (sorbitol)	−	−	−	0/46 (0)
L-Rhamnose	−	−	−	0/46 (0)
D-Trehalose	+	−	+	35/46 (76)
Citrate	−	−	−	0/46 (0)
Disk inhibition				
Polymyxin B (diameter of inhibition, >6 mm)[a]	+	+	+	46/46 (100)
Kanamycin (diameter of inhibition, ≥20 mm)[a]	+	−	+	17/46 (37)
Random isolates				
Arylsulfatase activity (3 days)	+	+	+	31/31 (100)
Semiquantitative catalase (≥100 mm)[a]	−[b]	ND[c]	−	17/17 (100)
Catalase (68°C)[a]	+	ND	+	14/19 (74)
Nitrate reduction[a]	−	ND	+	19/19 (100)
Iron uptake[a]	+	ND	+	18/19 (95)
MacConkey agar	ND	ND	+	17/17 (100)
Disk inhibition				
Cephalothin (diameter of inhibition, >6 mm)[a]	−	−	−	0/29 (0)

[a] First reported data for clinical isolates and for most tests for the ATCC type strain.
[b] For *M. porcinum* ATCC 33776^T, the semiquantitative catalase test outcome was weakly positive (the column of bubbles was 65 mm high), a result we considered negative.
[c] ND, not determined.

114); sequevar 4, accession number AY496140 (ATCC 49939); and sequevar 5, accession number AY496141 (*Mf*-205). The *hsp65* sequence for *M. neworleansense* ATCC 49404^T was submitted to GenBank as AY496143, and the sequence for *M. septicum* ATCC 700731^T was submitted as AY496142.

RESULTS

Growth and biochemical characteristics. All 48 isolates were negative for early and late pigmentation, grew at 30°C, grew in <7 days, and were susceptible to polymyxin B. They were typical of members of the *M. fortuitum* third biovariant sorbitol-negative group in that all isolates were positive for D-mannitol and i-*myo*-inositol carbohydrate utilization tests but negative for D-sorbitol (Table 2, All isolates section). Testing of a small number of isolates was done in 13 additional tests (Table 2, Random isolates section). This showed isolates also to be typical members of the *M. fortuitum* complex by virtue of having a positive arylsulfatase reaction at 3 days, growing on MacConkey agar without crystal violet, and being positive for nitrate reduction, iron uptake, and acetamide utilization (Table 2). The isolates were also negative for citrate, L-rhamnose, and D-trehalose.

These results matched those for *M. porcinum* ATCC 33776^T except the latter was nitrate negative as previously described (23). These are the first reported growth and biochemical data for a number of features (Table 2) for *M. porcinum* clinical isolates and for most of these tests for the *M. porcinum* ATCC type strain. Some growth and biochemical characteristics for 13 of these strains have been previously reported but were tested in a different laboratory (16).

β-Lactamase. β-Lactamase IEF patterns showed 9 of 14 (64%) isolates to exhibit β-lactamase pattern 2 and 12 of 14 (86%) isolates to exhibit pattern 2 or 4. All isolates with pattern 2 or 4 that were subjected to PRA belonged to this group. None of the isolates matched β-lactamase IEF pattern 3, which was shown to be present in the *M. fortuitum* third biovariant D-sorbitol-negative group in previous studies (28, 32).

FL-HPLC. Four clinical isolates of the selected PRA group of *M. fortuitum* third biovariant D-sorbitol-negative isolates were compared to *M. porcinum* ATCC 33776^T, *M. neworleansense* ATCC 49404^T, *M. intracellulare* 13950^T, *M. fortuitum* ATCC 6841^T, and *M. peregrinum* ATCC 14467^T. The isolates of the selected PRA group and *M. porcinum* yielded mycolic acid chromatograms that were typical of *Mycobacterium* species. All produced an indistinguishable pattern of two closely clustered sets of peaks (Fig. 1). This pattern was indistinguishable from the pattern produced by *M. fortuitum*, *M. peregrinum*, and *M. neworleansense*. It was separable from the pattern of the control strain of *M. intracellulare* (Fig. 1) as well as those of *M. mageritense* and members of the *M. smegmatis* group as reported previously (2, 26).

TABLE 3. Susceptibility results for isolates of the selected PRA group of the *M. fortuitum* sorbitol-negative third biovariant and *M. porcinum* ATCC 33776^T with the same PRA pattern[a]

Drug	MIC for *M. porcinum* ATCC 33776^T (μg/ml)	MICs for third biovariant isolates (μg/ml)						No. of clinical third biovariant isolates tested
		ATCC 49939	ATCC BAA-328	Clinical isolates				
				Range	MIC_{50}	MIC_{90}	Mode	
Group I (isolates from all time periods)								
Amikacin	≤2	4, 2	4	≤1–64	4	>32	32	39
Cefoxitin*	32	32	32	8–64	32	32	32	39
Ciprofloxacin	0.5	0.5, 1	0.125	0.25–1	0.5	1	0.5	39
Clarithromycin*	2	1	1	0.25–4	4	4	4	39
Doxycycline	4	8, 16	>32	≤0.12–128	16	>32	8, 32	39
Imipenem	8	4	≤0.5	1–8	4	8	4	39
Sulfamethoxazole	≤4	≤1, 2	≤1	≤2–8	2	8	≤1	39
Tobramycin*	2	4	32	≤1–>32	16	32	4	39
Group II (later isolates only)								
Gatifloxacin*	ND	ND	ND	≤0.12–0.25	≤0.12	≤0.12	≤0.12	16
Levofloxacin*	ND	ND	ND	≤0.25–1	0.5	1	0.25, 0.5	27
Linezolid*	4	ND	ND	1–8	4	8	4	26

[a] *, First reported data for clinical isolates and for most drugs for the ATCC reference strains. ND, not determined. MIC_{50}, concentration inhibitory for 50% of strains. MIC_{90}, concentration inhibitory for 90% of strains.

Susceptibility testing. Drug susceptibility tests using current NCCLS mycobacterial breakpoints (where available) showed the clinical isolates to be susceptible to ciprofloxacin, gatifloxacin, levofloxacin, sulfamethoxazole, and linezolid and intermediate or susceptible to cefoxitin, clarithromycin, imipenem, and amikacin (13). The isolates were intermediate or resistant to doxycycline except for 1 (2.6%) of 39 strains tested which was susceptible. The eight drugs tested against all 39 clinical isolates and the two reference isolates are shown in the Group I section of Table 3, while the three drugs tested against only a small number of isolates are shown in the Group II section of Table 3. The *M. porcinum* type strain, ATCC 33776, gave the same susceptibility pattern (Table 3). Susceptibilities to amikacin, ciprofloxacin, imipenem, doxycycline, and sulfamethoxazole for 13 of these isolates (*Mf*-91, *Mf*-131, *Mf*-147, *Mf*-239, *Mf*-450, *Mf*-485, *Mf*-487, *Mf*-533, *Mf*-607, *Mf*-611, *Mf*-661, *Mf*-771, and *Mf*-809) (determined in another laboratory) were published previously as part of an earlier taxonomy study (16). These are the first reported susceptibility data for seven antimicrobial agents for *M. porcinum* (Table 3), including the ATCC type strain, 33776.

PRA. By PRA of the *hsp65* gene, *M. porcinum* ATCC 33776^T and 49 of the 70 isolates of the third biovariant D-sorbitol-negative group exhibited the same restriction fragment length polymorphism pattern. They gave fragments of 235 and 210 bp with BstEII and 140, 125, and 100 bp with HaeIII. This pattern was unique from those of other currently recognized members of the *M. fortuitum* group (Fig. 2), with the exception of *M. septicum*. Enzyme restriction using BsaHI, however, distinguished the third biovariant isolates and the *M. porcinum* reference strain (260-, 100-, and 80-bp fragments) from *M. septicum* (260- and 80-bp fragments). Twenty-four of the clinical isolates were subjected to BsaHI digestion, and all gave the 260-, 100-, and 80-bp fragments of *M. porcinum*. PRA results of two ATCC isolates and two clinical isolates (ATCC 49939, ATCC BAA-328, *Mf*-131, and *Mf*-487) and *M. porcinum* ATCC 33776^T have been reported previously (16).

16S rRNA gene sequencing. Seventeen (37%) of the 46 clinical isolates (*Mf*-91, *Mf*-114, *Mf*-115, *Mf*-131, *Mf*-147, *Mf*-205, *Mf*-388, *Mf*-533, *Mf*-771, *Mf*-1568, *Mf*-1615, *Mf*-1847, *Mf*-1965, *Mf*-2106, *Mf*-2107, *Mf*-2108, and ATCC 49939) among the third biovariant D-sorbitol-negative group of isolates with the *M. porcinum* PRA pattern underwent partial 16S rRNA gene sequencing, and all exhibited 16S rRNA gene sequences identical to that of *M. porcinum* ATCC 33776^T over the first 500 bp beginning at the 5′ end. This region of the gene also shows 100% identity with *M. neworleansense* ATCC 49404^T (presently not included in the MicroSeq database), and therefore 12 clinical isolates (*Mf*-91, *Mf*-114, *Mf*-115, *Mf*-131, *Mf*-147, *Mf*-205, *Mf*-388, *Mf*-661, *Mf*-771 *Mf*-1568, *Mf*-1615, and *Mf*-2190) and ATCC 49939 were subjected to near-complete

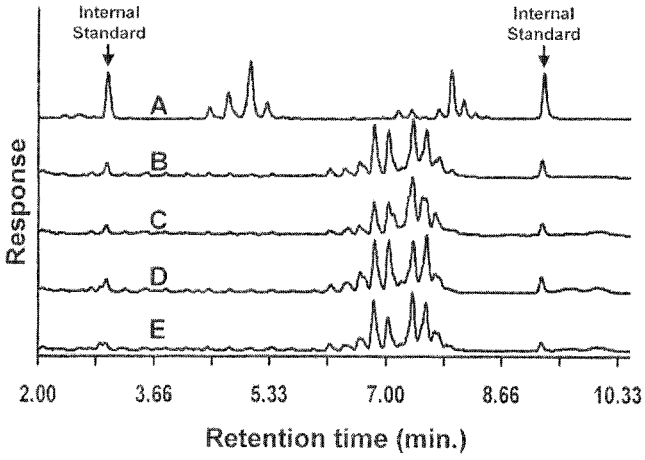

FIG. 1. Results of FL-HPLC with two strains of *M. porcinum* and three reference strains. The patterns of the last four isolates (B to D) are considered indistinguishable. (A) *M. intracellulare* ATCC 13950^T; (B) *M. porcinum* ATCC 33776^T; (C) *M. porcinum* ATCC 49939; (D) *M. peregrinum* type I ATCC 14467^T; (E) *M. fortuitum* ATCC 6841^T.

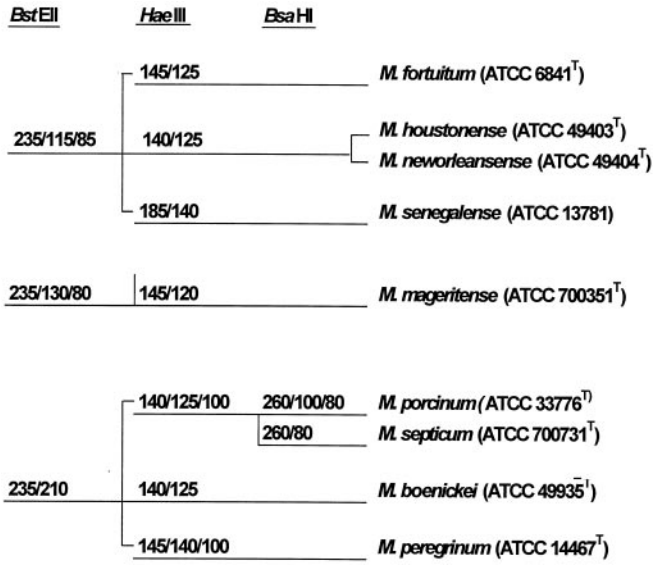

FIG. 2. PRA schema of the 441-bp Telenti fragment of the *hsp65* gene for separating members of the *M. fortuitum* group. An ATCC reference strain, where applicable, is included.

sequencing (1,463 bp) of the 16S rRNA gene and compared with the sequences previously determined for *M. neworleansense* ATCC 49404^T and *M. porcinum* ATCC 33776^T and ATCC 33775 (25). Ten of the 12 clinical third biovariant isolates and the reference isolates of *M. porcinum* had 100% sequence identity to each other, while one clinical strain (*Mf*-2190) differed by 1 bp from the other strains at *Escherichia coli* base-pair position 187. One isolate revealed a 1-bp variation from *M. neworleansense* at *E. coli* base-pair position 1135.

***hsp65* gene partial sequencing.** Twelve of the clinical *M. fortuitum* third biovariant D-sorbitol-negative isolates and both reference strains of *M. porcinum* were subjected to sequencing of the 441-bp Telenti fragment (*hsp65* gene). Four distinct sequence variants (sequevars) were observed among the clinical isolates, varying from each other by 1 to 2 bases. Both ATCC strains of *M. porcinum* were identical to each other and showed 2- to 3-bp variations from the clinical strains. The four clinical sequevars all contained a T→C substitution at positions 246 and 285 that was not present in the two porcine strains. None of these variations were in regions affected by restriction enzyme sites for BstEII, HaeIII, or BsaHI. The *hsp65* sequences of *M. septicum* ATCC 700731^T (with a PRA profile identical to that of *M. porcinum* as determined by use of BstEII and HaeIII) and *M. neworleansense* ATCC 49404^T (identical in the first 500 bp to the 16S rRNA gene sequence from *M. porcinum*) were also determined for comparison (Fig. 3). *M. neworleansense* differed by 11 bp, and *M. septicum* differed by 6 bp from the *M. porcinum* reference strains. Interestingly, both of these species also had the T→C substitution at positions 246 and 285.

DISCUSSION

These studies identified only a few minor phenotypic, drug susceptibility-related, or molecular differences between the human PRA group of the *M. fortuitum* third biovariant complex D-sorbitol-negative group and the porcine reference strains of *M. porcinum*. The original 10 strains of *M. porcinum*, characterized by Tsukamura et al., were found to be nitrate negative (23). The *M. porcinum* type strain was negative for nitrate reduction on repeat testing in the present study, while all third biovariant D-sorbitol-negative isolates tested, including the two ATCC strains, were positive for nitrate reduction (20 of 20 isolates; Tsukamura also tested 12 of these isolates in his laboratory, and all were positive). The type strain of *M. porcinum* had a low semiquantitative catalase (65 mm), while 17 of the 17 third biovariant isolates had a high (>100 mm) semiquantitative catalase. A small number of the third biovariant isolates were tested for benzoate as a sole carbon source in Tsukamura's laboratory, with 4 of 12 (33%) being positive compared to 100% positivity of the original published strains of *M. porcinum* (data not presented) (23). Other studies including drug susceptibilities, FL-HPLC, PRA, and carbohydrate utilization gave comparable results for the two groups.

The 16S rRNA gene sequence of the *M. fortuitum* third biovariant D-sorbitol-negative strain ATCC 49404 was first reported by Kirschner et al. in 1992 (11). This group subsequently sequenced hypervariable region A of the 16S rRNA gene of three additional third biovariant D-sorbitol-negative strains (ATCC 49935, ATCC 49937, and ATCC 49939) and found them identical to ATCC 49404 (19).

ATCC 49404 was found to have a PRA pattern of the *hsp65* gene that differed from those of the other third biovariant D-sorbitol-negative strains (20), although Turenne et al. recently showed *M. porcinum* ATCC 33776^T to differ from this strain by only 1 bp over the entire 1,400-bp 16S rRNA gene sequence (25). Schinsky et al. showed ATCC 49404 to have DNA-DNA homologies of <50% with all other taxa within the third biovariant group including *M. porcinum* and named this isolate *M. neworleansense* (16). No other third biovariant D-sorbitol-negative clinical strains were sequenced in the study by Turenne et al. (25). That group did complete sequences of other *M. fortuitum* group members, including *M. porcinum* ATCC 33776^T, and showed that the latter differed by 8 bp from its closest sequences. These were *M. farcinogenes* (a nonpigmented slow grower originally reported by Chamoiseau) (5, 15) and the proposed type strain of *M. houstonense*, ATCC 49403 (formerly of the *M. fortuitum* third biovariant D-sorbitol-positive group) (11, 16). Some of those differences are present in the first 500 bp of the 16S rRNA gene, and this sequence is presently different from those of all other recognized species.

Of the isolates of the *M. fortuitum* third biovariant D-sorbitol-negative group whose *hsp65* PRA patterns matched that of *M. porcinum* ATCC 33776^T, 4 isolates in the Schinsky et al. study (16) and 17 in the present study gave partial 16S rDNA gene sequences that matched that of the *M. porcinum* type strain. Eleven clinical isolates with almost complete 16S rRNA gene sequencing also showed 100% identity. This suggests that *hsp65* PRA will accurately identify isolates of *M. porcinum*.

The phenotype, drug susceptibility, and *hsp65* gene sequence similarity, the 16S rDNA identity, and the high degree of overall DNA homology (presented in the study by Schinsky et al.) (16) provide convincing evidence that the nonpigmented pig pathogen described in 1983 by Tsukamura et al. (23) is the same species as the present human pathogen previously grouped within the *M. fortuitum* third biovariant D-sorbitol-

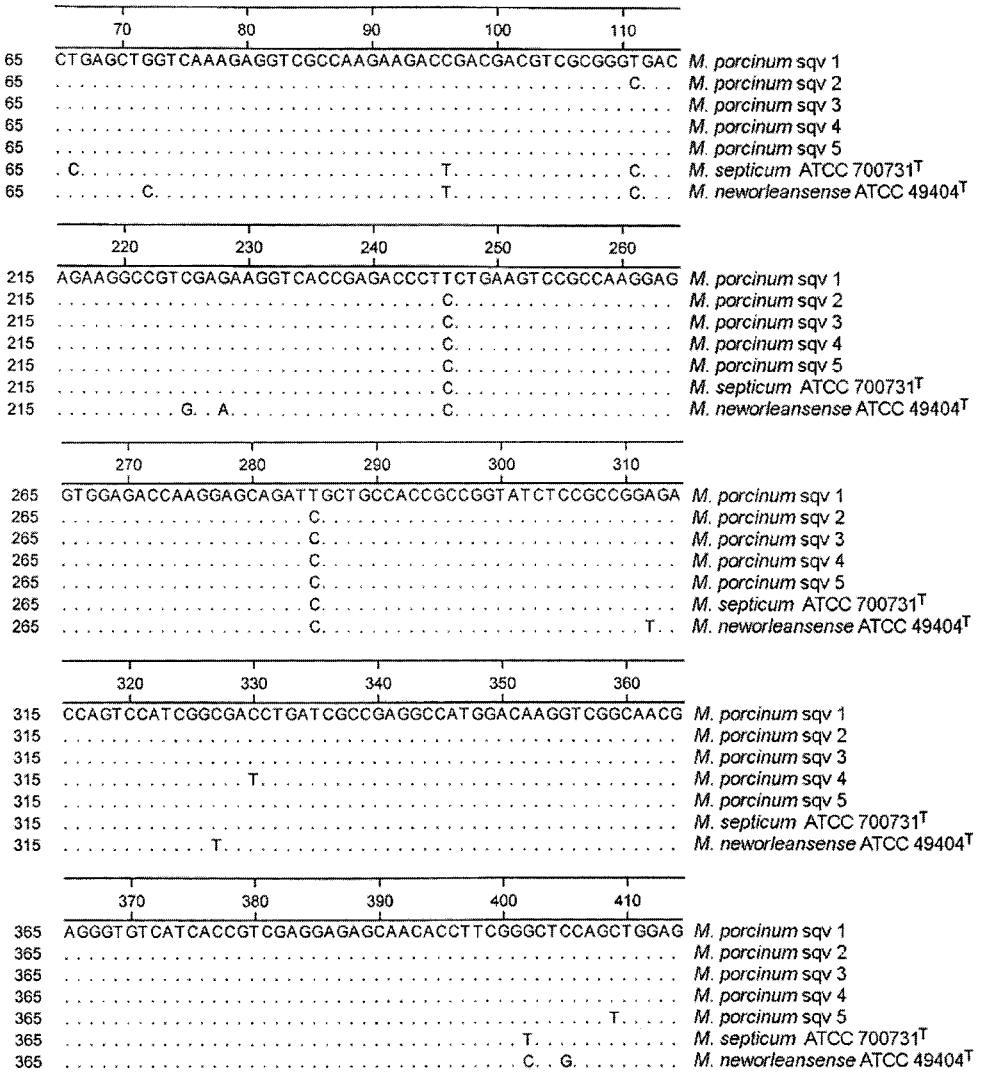

FIG. 3. Alignment of the partial *hsp65* gene of the various sequevars of the *M. porcinum* group and the closely related *M. septicum* ATCC 700731^T and *M. neworleansense* ATCC 49404^T. Base positions represent numbering for the 441-bp Telenti fragment. Regions not shown contain no variations. *M. porcinum* sequevar (sqv) 1, *M. porcinum* ATCC 33776^T and *M. porcinum* ATCC 33775; *M. porcinum* sqv 2, clinical strains *Mf*-2190, *Mf*-131, *Mf*-147, *Mf*-388, *Mf*-115, ATCC BAA-328 (*Mf*-771), *Mf*-1568, and *Mf*-91; *M. porcinum* sqv 3, *Mf*-1615 and *Mf*-114; *M. porcinum* sqv 4, ATCC 49939; *M. porcinum* sqv 5, *Mf*-205.

negative group (27). We would agree with calling these isolates *M. porcinum*, with three reference strains (one porcine, two human) presently catalogued in the ATCC.

The sources and clinical disease associated with these isolates of *M. porcinum* clearly define them as human pathogens. Their role in causing chronic lung infection is not well established, but there is little question of their ability to cause posttraumatic or postsurgical wound infections, osteomyelitis, and catheter-related infections. These findings are similar to what has been reported for all members of the *M. fortuitum* third biovariant group (27). As has been noted with several types of surgical wound infections, the *M. porcinum* clinical isolates submitted to us are concentrated among southern coastal states (6, 12, 29, 30).

This study identified two environmental isolates of *M. porcinum* (*Mf*-1615 and *Mf*-1616), the first of these to be reported. Both were recovered from tap water (8). This reservoir could explain the frequent association of this species with catheter-related infections, since catheter exposure to tap water probably occurs frequently.

M. porcinum isolates are phenotypically separable from isolates of the three recognized or proposed species of the *M. fortuitum* third biovariant D-sorbitol-positive group using L-rhamnose (*M. mageritense*), D-sorbitol (*M. mageritense*, *M. houstonense*, and *M. brisbanense*), and clarithromycin susceptibility (same three species) (Table 4). It is not phenotypically separable from any of the other four presently recognized species or species of the *M. fortuitum* third biovariant D-sorbitol-negative group (*M. septicum*, *M. neworleansense*, *M. concordense*, and *M. boenickei*) (Table 4). All four species have the same antibiotic susceptibility pattern, including being intermediate or susceptible to cefoxitin and clarithromycin. This com-

TABLE 4. Useful tests for separating *M. porcinum* from other members of the *M. fortuitum* group[d]

Species (strain) (reference)	Antibiotic resistance		Carbohydrate utilization				Low catalase (<100 mm)	Unique PRA *hsp65* gene	Unique 16S r-DNA		
	Clari (MIC, >4 μg/ml)	Fox (MIC, ≥128 μg/ml)	D-man	i-*myo*-inos	D-sorb	L-rham			Hyper-variable A region	First 500 bp (5′ end)	Entire sequence
M. fortuitum (ATCC 6841^T) (4)	Var	−	−	−	−	−	−	+	+	+	+
M. neworleansense (ATCC 49404^T) (16, 27)	−	−	+	+	−	−	−	−	−[a]	−	+
M. mageritense (ATCC 700351^T) (26)	+	−	+	+	+	+	+	+	+	+	+
M. porcinum (ATCC 33776^T, BAA-328, ATCC 49939)	−	−	+	+	−	−	−	+	−[a]	−	+
M. septicum (ATCC 700731^T) (17)	−	−	+	+	−	−	−	+	−[a,b]	−[b]	−[b]
M. houstonense (ATCC 49403^T) (4, 16, 27)	+	+	+	+	+	−	+	−	−[c]	−[c]	−[c]
M. boenickei (ATCC 49935^T) (16)	−	−	+	+	−	−	−	+	−[a]	NK	+
M. peregrinum type I (ATCC 14467^T) (4)	−	−	+	−	−	−	−	+	−[b]	−[b]	−[b]
M. senegalense (ATCC 35796^T)	−	−	+	−	−	−	−	+	−[c]	−[c]	+

[a] *M. porcinum* and other members of the *M. fortuitum* third biovariant sorbitol-negative group, other than *M. septicum*, have the same hypervariable A sequence.
[b] *M. septicum* and *M. peregrinum* type I have the same sequence over the entire 1,400-bp 16S rRNA gene sequence.
[c] *M. senegalense, M. houstonense*, and *M. farcinogenes* have the same sequence over the first 500 bp. *M. houstonense* and *M. farcinogenes* are also identical over the entire 1,400 bp.
[d] An ATCC reference strain, where applicable, is included. Abbreviations: Var, variable; Clari, clarithromycin; Fox, cefoxitin; man, mannitol; inos, inositol; sorb, sorbitol (D-glucitol); rham, rhamnose; +, ≥90% positive for indicated characteristic; −, ≤10% negative for indicated characteristic; NK, not known.

ment is qualified by the observation that only a single strain each of *M. septicum*, *M. neworleansense*, and *M. concordense* has been reported and characterized to date (16, 17).

The best biochemical screening test for selection of *M. fortuitum* among isolates belonging to the *M. fortuitum* group would appear to be D-mannitol. D-Mannitol-negative isolates are presumptive *M. fortuitum*, while D-mannitol-positive isolates are not *M. fortuitum* but require additional testing to define the species. Sequencing of the hypervariable A region of the 16S rRNA gene or even the first 500 bp results in a great deal of overlap for numerous taxa (25). This region identifies *M. porcinum* and *M. neworleansense* as distinct from the rest of the *M. fortuitum* complex but identical to each other. A single additional sequencing reaction encompassing the V7 region of the 16S rRNA gene can differentiate between these two species due to a 1-bp variation at position 1135 and could therefore provide accurate species identification. These species are better distinguished by their *hsp65* sequences, showing 11 mismatches or a 97.5% sequence identity, contributing to clearly distinct PRA patterns. The best single molecular test for identifying species within the *M. fortuitum* group would appear to be *hsp65* PRA, as it clearly separated the sorbitol-positive species and *M. boenickei* from *M. porcinum* within the sorbitol-negative group.

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