Mycobacterium porcinum sp. nov., a Porcine Pathogen

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Ten strains of rapidly growing, non-photochromogenic mycobacteria were isolated from submandibular lymph nodes with tuberculosis-like lymphadenitis of 10 swine. These mycobacteria showed a positive reaction for arylsulfatase activity after 3 days and resistance to $\text{NH}_2\text{OH} \cdot \text{HCl}$ (0.5 mg/ml) and degraded *p*-aminosalicylate, forming black formazan. These strains were similar to *Mycobacterium fortuitum*, but differed from this species by lacking nitrate reduction activity, having positive succinamidase activity, and having the ability to utilize benzoate as a sole source of carbon in the presence of ammoniacal nitrogen. These mycobacteria were considered to belong to a new species, *Mycobacterium porcinum* sp. nov. The type strain was deposited in the American Type Culture Collection as strain ATCC 33776 (= E10241-1).

In 1973, Tsukamura and Nemoto (5) studied the biological characteristics and serotypes of *Mycobacterium intracellulare* strains isolated from submandibular lymph nodes of swine. A total of 25 strains of mycobacteria were isolated from lymph nodes showing tuberculosis-like lymphadenitis of different animals. Of these, 21 belonged to *M. intracellulare*. The remaining four were rapidly growing, non-photochromogenic mycobacteria. Of these four, three were identified as *Mycobacterium fortuitum*, and one was believed to belong to a new species. However, this single isolate was not named at that time. Subsequently, we isolated the same organism from the lymph nodes of nine other swine. Ten strains of this organism were studied. In the present study, the characteristics of these strains are described, and the species is designated *Mycobacterium porcinum* sp. nov.

MATERIALS AND METHODS

Test strains were isolated from submandibular lymph nodes with tuberculosis-like lymphadenitis by inoculating lesion material onto Ogawa egg medium. The isolates were lyophilized until examination. Nine additional strains of rapidly growing, non-photochromogenic mycobacteria belonging to another species were also isolated from the lymph nodes. These also were examined in this study. The new isolates were compared with the type strains of all known rapidly growing mycobacteria.

The characters tested and the methods used were those described previously (4).

The characters of the test strains were compared numerically by using the matching coefficient (M value). The M value between any two strains was calculated by the following equation: $M \text{ value} = (n_s \times 100\%) / (n_s + n_d)$, where n_s is the number of characters that show similar code symbols and n_d is the number of characters that show different code symbols. The range of values for a species was expressed as follows:

$M \pm 2SD$, where M is the mean of M values estimated for the strains of the species compared with the hypothetical median organism pattern (HMO) of the species and SD is the standard deviation (3).

RESULTS AND DISCUSSION

A total of 19 strains isolated from porcine lymph nodes were divided into two distinct groups, and both of these groups were very homogeneous. The 10 strains of *M. porcinum* sp. nov. and the 9 strains identified as *M. fortuitum* had the following similar properties: all were acid-fast, short or long rods; all produced neither permanent nor fragmenting mycelium; all showed no cross-barring or cord formation; none produced colony pigmentation in the dark or after exposure to light; all grew at 28 and 37°C but not at 45 and 52°C; all grew on Ogawa egg medium and on Löwenstein-Jensen egg medium in 3 days; all grew on modified Sauton agar medium after 3 days; all grew on modified Sauton agar media containing 1 mg of salicylate per ml, 0.1% picric acid, 0.2% picric acid, 0.1% NaNO_2 , 0.2% NaNO_2 , and 1% Tween 80; all were resistant to *p*-aminosalicylate (2 mg/ml), $\text{NH}_2\text{OH} \cdot \text{HCl}$ (125, 250, and 500 µg/ml), rifampin (25 µg/ml), ethambutol (5 µg/ml), salicylate (0.5 mg/ml), and *p*-nitrobenzoic acid (0.5 mg/ml).

In addition, all strains degraded *p*-aminosalicylate to catechol, formed black formazan, and were arylsulfatase positive after 3 days, as well as after 14 days; niacin was not produced; Tween 80 was not hydrolyzed after 7 and 14 days. All strains were catalase positive (foam height more than 45 mm), β -galactosidase negative, β -esterase positive, acid phosphatase negative, acetamidase positive, urease positive, nicotinamidase positive, pyrazinamidase positive, allantoinase positive, benzamidase negative,

isonicotinamidase negative, and salicylamidase negative. Glutamate, glucosamine, acetamide, monoethanolamine, and trimethylenediamine were utilized as simultaneous nitrogen and carbon sources, but benzamide was not. The following compounds were utilized as sole carbon sources in the presence of ammoniacal nitrogen: acetate, succinate, malate, pyruvate, fumarate, glucose, fructose, mannose, ethanol, *n*-propanol, *n*-butanol, isobutanol, and 2,3-butylene glycol. The following compounds were not utilized as sole carbon sources in the presence of ammoniacal nitrogen: malonate, 1,4-butylene glycol, galactose, sucrose, arabinose, xylose, rhamnose, and sorbitol. Acid was formed from glucose and mannose. The following compounds were utilized as sole nitrogen sources in the presence of glycerol carbon: acetamide, urea, pyrazinamide, nicotinamide, and nitrate.

The reactions that differed among strains within species or between the two species are shown in Table 1. All 19 strains showed three or all four of the distinguishing characteristics of the *Mycobacterium fortuitum* complex, *M. fortuitum*, and *Mycobacterium chelonae* (1, 2, 6) (Table 2).

Of the 19 strains described above, 9 were identified as *M. fortuitum*, and these strains showed M values of 93 or 94% compared with the HMO of *M. fortuitum*, which was determined previously for 78 strains isolated from different sources. The M values of these nine isolates were within the range of values for *M. fortuitum* ($93.3 \pm 5.2\%$). These nine strains were more homogeneous with respect to several characters than the 78 strains collected from different sources (Table 2).

Ten strains from lymph nodes were considered to belong to a new species. The mean M value for these 10 strains compared with their HMO was $98.6 \pm 0.7\%$, which shows that this taxon is very homogeneous.

The mean M value for the *M. fortuitum* strains isolated from lymph nodes in this study compared with their HMO was $97.7 \pm 1.2\%$. The M value between the two HMOs was 87.5%. Thus, the two taxa which we studied were considered to be distinct. The separate taxon was named *Mycobacterium porcinum* sp. nov., and strain E10241-1 was selected as the type strain. The M values of this type strain compared with the type strains of all rapidly growing mycobacteria were determined (Table 3). The highest M value (85%) was obtained with the type strain of *M. fortuitum*.

To confirm the distinction between *M. porcinum* and *M. fortuitum*, we estimated M values for the strains of each species compared with the HMO of the same species and compared with the HMO of the other species. The mean M

TABLE 1. Differential and variable characteristics among 10 *M. porcinum* strains and 9 *M. fortuitum* strains isolated from porcine lymph nodes

Character ^a	% Of strains positive	
	<i>M. porcinum</i> (10 strains)	<i>M. fortuitum</i> (9 strains)
Long rods (more than 7 μ m long)	10	11
Intermediate rods (3–6 μ m long)	50	56
Rough colonies	40	44
Degradation of salicylate to form black formazan	0	100
α -Esterase	50	0
Nitrate reduction (6 h)	0	100
Nitrate reduction (24 h)	0	100
Succinamidase	100	0
Serine used as simultaneous N and C sources	100	11
Citrate used as sole C source	100	0
Benzoate used as sole C source	100	0
Propylene glycol used as sole C source	100	11
1,3-Butylene glycol used as sole C source	100	67
Trehalose used as sole C source	100	67
Inositol used as sole C source	100	0
Mannitol used as sole C source	100	0
Benzamide used as sole N source	100	0
Nitrite used as sole N source	100	33

^a Unless otherwise noted, resistance to each agent was tested in Ogawa egg medium, the utilization of carbohydrates as sole carbon sources was tested in the presence of ammoniacal nitrogen, and the utilization of nitrogen compounds as sole nitrogen sources was tested in the presence of glycerol carbon.

value of the 78 strains of *M. fortuitum* compared with the HMO of *M. porcinum* was $85.8 \pm 2.3\%$, whereas the mean M value of the 10 strains of *M. porcinum* compared with the *M. porcinum* HMO was $98.6 \pm 0.7\%$. The lower limit of the range of values for *M. porcinum*, 97.2%, was significantly higher than upper limit of the range of values for *M. fortuitum*, 90.4%. Thus, these two species were considered to be distinct, as they showed no overlap.

Compared with the HMO of *M. fortuitum*, the mean M value of the 10 strains of *M. porcinum* was $88.2 \pm 0.6\%$, and the mean M value of the 78 strains of *M. fortuitum* was $93.3 \pm 2.6\%$. Thus, the lower limit of the range of values for *M. fortuitum* was estimated to be 88.1%, and the upper limit of the range of values for *M. por-*

TABLE 2. Characters distinguishing *M. porcinum*, *M. fortuitum*, *M. chelonae* subsp. *abscessus*, and *M. chelonae* subsp. *chelonae*^a

Character	% Of strains positive			
	<i>M. porcinum</i> (10 strains)	<i>M. fortuitum</i> (78 strains)	<i>M. chelonae</i> subsp. <i>abscessus</i> (14 strains)	<i>M. chelonae</i> subsp. <i>chelonae</i> (12 strains)
Arylsulfatase (3 days) ^b	100	94	93	100
Resistance to NH ₂ OH·HCl (0.5 mg/ml) ^b	100	100	100	100
Degradation of <i>p</i> -aminosalicylate ^b	100	65	100	100
Degradation of salicylate (Sauton agar) ^b	0	67	93	58
Tolerance to 0.2% picric acid (Sauton agar)	100	99	93	8
β-Galactosidase	0	17	86	100
Nitrate reduction (24 h)	0	100	14	0
Acetamidase	100	87	14	8
Allantoinase	100	99	0	0
Succinamidase	100	0	0	0
Acetamide used as N and C sources	100	56	7	0
Trimethylenediamine used as N and C sources	100	100	86	0
Citrate used as C source	100	18	7	100
Benzoate used as C source	100	3	0	0
Ethanol used as C source	100	91	0	0
<i>n</i> -Propanol used as C source	100	100	14	0
Propylene glycol used as C source	100	78	0	0
1,3-Butylene glycol used as C source	100	71	0	0
2,3-Butylene glycol used as C source	100	60	0	0
<i>n</i> -Butanol used as C source	100	100	79	0
Inositol used as C source	100	13	0	0
Mannitol used as C source	100	53	7	0
Benzamide used as N source	100	5	0	0
Nitrite used as N source	100	73	93	0

^a The data for *M. fortuitum* and *M. chelonae* were taken from a previous study (4).

^b These characteristics do not distinguish among the species shown on the table, but do distinguish these species from other mycobacteria. Three or four positive results for these four characteristics indicate that a test strain belongs to *M. fortuitum*, *M. chelonae*, or *M. porcinum*.

cinum was estimated to be 89.4%. There was little overlap between the distribution of the M values for these two taxa compared with the HMO of *M. fortuitum*. However, since a distinct separation between the two species was confirmed on one side, these two taxa may be regarded as different.

A comparison of the characteristics of *M. porcinum*, *M. fortuitum*, and *M. chelonae* is shown in Table 2. *M. porcinum* is differentiated from *M. fortuitum* by the following characteristics of *M. porcinum*: negative nitrate reduction activity; positive succinamidase activity; benzoate utilized as a sole carbon source in the presence of ammoniacal nitrogen; citrate utilized as a sole carbon source in the presence of ammoniacal nitrogen; inositol utilized as a sole carbon source in the presence of ammoniacal nitrogen; benzamide utilized as a sole nitrogen source in the presence of glycerol carbon.

Unlike *M. chelonae*, *M. porcinum* has the following properties: positive for acetamidase; positive for allantoinase; positive for succinamidase; acetamide utilized as simultaneous nitrogen and carbon sources; trimethylenediamine utilized as

simultaneous nitrogen and carbon sources; benzoate utilized as a sole carbon source; ethanol utilized as a sole carbon source; *n*-propanol utilized as a sole carbon source; propylene glycol utilized as a sole carbon source; *n*-butanol utilized as a sole carbon source; isobutanol utilized as a sole carbon source; 1,3-butyleneglycol utilized as a sole carbon source; 2,3-butyleneglycol utilized as a sole carbon source.

Until now, the succinamidase activity and the ability to utilize benzoate as a sole carbon source have been observed only in *Mycobacterium smegmatis*. However, *M. smegmatis* is differentiated from *M. porcinum* by the following characteristics: growth at 45°C; positive for nitrate reduction; benzamidase and isonicotinamidase activities; utilization of malonate, galactose, arabinose, xylose, rhamnose, and sorbitol as sole carbon sources (4).

Description of *Mycobacterium porcinum* sp. nov. (por'ci-num. L. adj. *porcinum* pertaining to swine). The characteristics of the type strain are as follows. Acid-fast rods (1.5 to 6 µm long by 0.5 µm wide); colonies on egg media are smooth to rough and non-photochromogenic,

TABLE 3. Comparison of the type strain of *M. porcinum* (strain E10241-1) with the type strains of previously described species and subspecies of rapidly growing mycobacteria

Species or subspecies	Strain	M value (%) of strain E10241-1 ^T compared with each type strain
<i>M. fortuitum</i>	ATCC 6841 ^T	85
<i>M. chelonae</i> subsp. <i>chelonae</i>	NCTC 946 ^{Ta}	71
<i>M. chelonae</i> subsp. <i>abscessus</i>	ATCC 19977 ^T	79
<i>M. smegmatis</i>	ATCC 14468 ^b	78
<i>M. chitae</i>	ATCC 19627 ^T	65
<i>M. agri</i>	ATCC 27406 ^T	67
<i>M. farcinogenes</i>	NCTC 10955 ^{Tc}	48
<i>M. senegalense</i>	NCTC 10956 ^{Tc}	73
<i>M. flavescens</i>	ATCC 14474 ^T	64
<i>M. thermoresistibile</i>	ATCC 19527 ^T	62
<i>M. phlei</i>	ATCC 11758 ^T	69
<i>M. vaccae</i>	ATCC 15483 ^T	63
<i>M. parafortuitum</i>	ATCC 19686 ^T	76
<i>M. aurum</i>	ATCC 23366 ^T	75
<i>M. neoaurum</i>	ATCC 25795 ^T	81
<i>M. rhodesiae</i>	ATCC 27024 ^T	77
<i>M. obuense</i>	ATCC 27023 ^T	73
<i>M. aichiense</i>	ATCC 27280 ^T	71
<i>M. chubuense</i>	ATCC 27278 ^T	70
<i>M. tokaiense</i>	ATCC 27282 ^T	69
<i>M. duvalii</i>	NCTC 358 ^{Ta}	64
<i>M. gilvum</i>	NCTC 10742 ^{Ta}	73
<i>M. gadium</i>	ATCC 27726 ^T	75
<i>M. sphagni</i>	ATCC 33027 ^T	62
<i>M. komossense</i>	ATCC 33013 ^T	69
" <i>M. austroafricanum</i> "	ATCC 33464	74

^a Received from J. L. Stanford, Middlesex Hospital Medical School, London, England.

^b Reference strain.

^c Received from M. P. Lechevalier, Rutgers University, New Brunswick, N.J.

and colonies on modified Sauton agar medium (2) are rough and dry. Growth occurs after 3 days on egg media and at 28, 37, and 42 but not 45°C. Resistant to the following agents (on Ogawa egg medium or Löwenstein-Jensen medium): *p*-nitrobenzoic acid (0.5 mg/ml), 5% NaCl, thiophene-2-carboxylic acid hydrazide (1 µg/ml), rifampin (25 µg/ml), ethambutol (5 µg/ml), sodium *p*-aminosalicylate (2 mg/ml), sodium salicylate (0.5 mg/ml), and hydroxylamine hydrochloride (0.5 mg/ml). Tolerant to 0.2% picric acid, 0.2% sodium nitrite, and 0.1% sodium salicylate on modified Sauton agar medium. Degrades *p*-aminosalicylate to form black formazan, but does not degrade salicylate. Niacin

is not produced. Tween 80 is not hydrolyzed after 14 days. Nitrate is not reduced to nitrite after 24 h. Arylsulfatase positive after 3 days; catalase positive (foam height more than 100 mm); β-galactosidase negative; acid phosphatase negative; β-esterase positive, but α-esterase negative or, if not negative, weakly positive. Amidase pattern: acetamidase, urease, nicotinamidase, pyrazinamidase, allantoinase, and succinamidase positive; benzamidase, isonicotinamidase, salicylamidase, and malonamidase negative. Pattern of utilization of nitrogen compounds as simultaneous nitrogen and carbon sources: glutamate, serine, glucosamine, acetamide, monoethanolamine, and trimethylenediamine are utilized; benzamide is not utilized. Pattern of utilization of carbohydrates as sole sources of carbon in the presence of ammoniacal nitrogen: acetate, citrate, succinate, malate, pyruvate, benzoate, fumarate, ethanol, *n*-propanol, *n*-butanol, isobutanol, propylene glycol, 1,3-butylene glycol, 2,3-butylene glycol, glucose, fructose, mannose, trehalose, inositol, and mannitol are utilized; malonate, 1,4-butylene glycol, sucrose, galactose, arabinose, xylose, rhamnose, and sorbitol are not utilized. Acetamide, benzamide, urea, nicotinamide, pyrazinamide, nitrate, and nitrite are utilized as sole nitrogen sources in the presence of glycerol carbon.

The nine other strains examined had identical characteristics, except that one produced cells longer than 7 µm and six produced smooth colonies on egg media (however, all 10 strains formed rough dry colonies on modified Sauton agar medium).

The source of all 10 strains was the lymph nodes of swine which showed tuberculosis-like lymphadenitis. The type strain is E10241-1 (= ATCC 33776).

LITERATURE CITED

1. Tsukamura, M. 1970. Differentiation between *Mycobacterium abscessus* and *Mycobacterium borstelense*. *Am. Rev. Respir. Dis.* 101:426-428.
2. Tsukamura, M. 1975. Identification of mycobacteria. The National Chubu Hospital, Obu, Aichi, Japan.
3. Tsukamura, M. 1976. An approach to numerical identification of bacterial species. *J. Gen. Microbiol.* 95:207-212.
4. Tsukamura, M. 1981. Numerical analysis of rapidly growing, nonphotochromogenic mycobacteria, including *Mycobacterium agri* (Tsukamura 1972) Tsukamura sp. nov., nom. rev. *Int. J. Syst. Bacteriol.* 31:247-258.
5. Tsukamura, M., and H. Nemoto. 1973. A taxonomic study of *Mycobacterium intracellulare* isolated from swine. *Jpn. J. Microbiol.* 17:91-98.
6. Tsukamura, M., and S. Tsukamura. 1967. A practical system for identification of *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium kansasii*, and *Mycobacterium fortuitum*. *Scand. J. Respir. Dis.* 48:58-70.