Mycobacterium shimoidei sp. nov., nom. rev., a Lung Pathogen

MICHIO TSUKAMURA

National Chubu Hospital, Obu, Aichi, Japan 474

"*Mycobacterium shimoidei*" Tsukamura, Shimoide, and Schaefer 1975 was not included on the Approved Lists of Bacterial Names and has not been validly published since 1 January 1980; hence, it does not have standing in bacterial nomenclature. The organism is here regarded as a distinct species of the genus *Mycobacterium*, and the name *Mycobacterium shimoidei* is revived for the same organism to which this name was originally applied. The type strain is strain E4796 (= ATCC 27962).

"*Mycobacterium shimoidei*" Tsukamura, Shimoide, and Schaefer 1975 (8) was isolated from a lung infection in a Japanese patient. This organism differed from all other slowly growing mycobacteria known at the time (6). However, this name did not appear on the Approved Lists of Bacterial Names (4), and it has not been validly published since 1 January 1980; thus, it is without standing in bacterial nomenclature. In 1981, the International Working Group on Mycobacterial Taxonomy (9) supported revival of the name "*M. shimoidei*," citing another case of lung infection due to this organism, this time from Australia. The purpose of the present paper is to revive the name "*M. shimoidei*" for the same taxon with which the name was originally associated.

MATERIALS AND METHODS

Bacterial strains. Four strains of "*M. shimoidei*" (E4794, E4796 [= ATCC 27962], E7367, and E7380), 3 strains of *M. ulcerans* (RM, OBJ, and POW), 1 strain of *M. haemophilum* (No. 1 [= ATCC 29548], the type strain), and 15 strains, including the type strain (ATCC 29571), of *M. malmoense* (1) were used. The strains of "*M. shimoidei*" were identified in the Research Laboratory of the National Chubu Hospital; those of *M. ulcerans* were received from D. J. Dawson, Laboratory of Microbiology and Pathology, Brisbane, Australia; the strain of *M. haemophilum* was received from D. Sompolinsky, Asaf Harofe Hospital, Zerfin, Israel; and the strains of *M. malmoense* were from P. A. Jenkins, Mycobacterium Reference Laboratory, University Hospital of Wales, Cardiff, Wales. The other strains of slowly growing mycobacteria studied were cited in a previous paper (6).

Tests. Eighty-eight characters were determined for each strain. These were listed and described previously (6).

Numerical taxonomy. The hypothetical median organism (HMO) was determined for the strains of each species (2). For the strains of each species, matching coefficients (*M* values) were determined against the

HMO of the species, and the range of a species was regarded as $M \pm 2s$ (percent), where *M* is the mean of *M* values for the strains of each species against the HMO of the species and *s* is its standard deviation. Therefore, the lower limit of the range is $M - 2s$ (percent) (7).

RESULTS AND DISCUSSION

M values of strain ATCC 27962 of "*M. shimoidei*" to the HMO of this species and to the HMOs of other slowly growing mycobacteria were determined (Table 1). The *M* values of ATCC 27962 to the HMOs of the other species were outside the low limit of the species, whereas the *M* value to the HMO of "*M. shimoidei*" was higher than the lower limit of the species. Thus, it is considered that "*M. shimoidei*" is distinct from all other species of slowly growing mycobacteria. Characters useful for distinguishing "*M. shimoidei*" from all other slowly growing mycobacteria were previously reported by Tsukamura et al. (8), Tsukamura (6), and Wayne et al. (9). Characters useful in differentiating "*M. shimoidei*" from two recently described species, *M. malmoense* (3) and *M. haemophilum* (5), and from *M. ulcerans* are shown in Table 2. The name "*M. shimoidei*" is here revived for the same organism with which the name was originally associated.

Mycobacterium shimoidei sp. nov., nom. rev. shimo.idé.i. M.L. gen. n. *shimoidei* of Shimoide; named for H. Shimoide, the first to isolate this organism.

Acid-fast rods, 0.5 μ m by 3.0 to 5.0 μ m; cross-barring ordinarily occurs; cords are not formed; mycelium is not produced.

Forms rough colonies which are not pigmented when grown in the dark or when exposed to light (non-photochromogenic); grows on egg media (Ogawa egg medium and Löwenstein-Jensen medium) after incubation at 37°C for 14 to 21

TABLE 1. Differentiation of *M. shimoidei* from other species of slowly growing mycobacteria on the basis of numerical taxonomy results

Species	No. of strains tested	Mean $\pm$ SD of matching coefficients for the strains of a species against the HMO of the species: $M \pm s$ (%)	Lower limit of the range of M values: $M - 2s$ (%) ^a	M value (%) of the type strain of <i>M. shimoidei</i> to the HMOs of other species
<i>M. tuberculosis</i>	9	99.11 $\pm$ 0.93	97.25	80.7
<i>M. bovis</i>	12	97.00 $\pm$ 1.54	93.92	83.0
<i>M. africanum</i>	11	97.36 $\pm$ 1.63	94.10	84.1
<i>M. microti</i>	6	97.17 $\pm$ 2.23	92.71	77.3
<i>M. kansasii</i>	11	95.91 $\pm$ 2.12	91.67	76.1
<i>M. marinum</i>	14	95.50 $\pm$ 2.50	90.50	77.3
<i>M. scrofulaceum</i>	8	96.13 $\pm$ 2.59	90.95	77.3
<i>M. xenopi</i>	4	96.75 $\pm$ 1.26	94.23	81.8
<i>M. szulgai</i>	6	97.33 $\pm$ 1.21	94.91	83.0
<i>M. gordonae</i>	12	95.08 $\pm$ 3.00	89.08	73.9
<i>M. avium</i>	7	98.00 $\pm$ 1.53	94.94	85.2
<i>M. intracellulare</i>	18	95.44 $\pm$ 2.48	90.48	77.3
<i>M. simiae</i>	5	99.00 $\pm$ 0.71	97.58	73.9
<i>M. asiaticum</i>	4	97.00 $\pm$ 2.94	91.12	79.5
<i>M. nonchromogenicum</i>	8	97.00 $\pm$ 2.67	91.66	78.4
<i>M. terrae</i>	9	95.33 $\pm$ 3.74	88.97	86.4
<i>M. triviale</i>	6	95.83 $\pm$ 2.48	90.87	85.2
<i>M. gastri</i>	8	97.13 $\pm$ 3.09	90.95	77.3
<i>M. malmoense</i>	15	96.33 $\pm$ 1.53	93.27	84.1
<i>M. ulcerans</i>	3	99.33 $\pm$ 0.58	98.17	88.6
<i>M. haemophilum</i>	1			81.8
<i>M. shimoidei</i> ^b	4	98.50 $\pm$ 1.29	95.92	98.9

^a About 95% of the strains of each species are expected to have M values higher than the lower limit (7).^b The four strains of *M. shimoidei* were isolated from a single patient.

days (when inoculated as single cells onto egg media, growth occurs after 21 or more days); does not grow on Sauton agar or on Sauton agar containing 0.1% NaNO₂ or 0.2% picric acid (no growth on Sauton agar containing 0.1% NaNO₂ or 0.2% picric acid shows that the test strains belong to the group of slowly growing mycobacteria [6]); growth at 28°C is variable; growth occurs at 37 and 45°C but not at 52°C. Grows on Ogawa egg medium (or Löwenstein-Jensen medium) containing 0.2% sodium *p*-aminosalicylate, rifampin (25 µg/ml), *p*-nitrobenzoic acid (0.5 mg/ml), sodium salicylate (0.5 mg/ml), or thiophene-2-carboxylic acid hydrazide (10 µg/ml); does not grow on Ogawa egg medium containing ethambutol (5 µg/ml), NH₂OH·HCl (0.25 mg/ml), 5% NaCl, or isoniazid (10 µg/ml). Niacin is not produced; Tween is not hydrolyzed after 7 days but is hydrolyzed after 14 days (strains maintained in laboratories hydrolyze Tween after 7 days); α-esterase negative; β-esterase negative; β-galactosidase negative; acid phosphatase positive; catalase (semiquantitative) negative; nitrate not reduced to nitrite after 24 h; arylsulfatase negative after 14 days; nico-

tinamidase and pyrazinamidase positive (these may be negative with strains tested immediately after isolation from sputum); acetamidase, benzamidase, urease, isonicotinamidase, salicylamidase, allantoinase, and succinamidase negative. The following carbohydrates are not utilized as a sole source of carbon in the presence of ammoniacal nitrogen: acetate, citrate, succinate, malate, pyruvate, benzoate, malonate, fumarate, glucose, fructose, sucrose, mannose, galactose, arabinose, xylose, rhamnose, trehalose, inositol, mannitol, sorbitol, ethanol, *n*-propanol, *n*-butanol, isobutanol, propylene glycol, and 1,3-, 2,3-, and 1,4-butylene glycols. The following nitrogen compounds are not utilized as a sole source of nitrogen in the presence of glycerol: glutamate, serine, methionine, acetamide, benzamide, urea, pyrazinamide, nicotinamide, isonicotinamide, succinamide, nitrate, and nitrite. L-Glutamate is not utilized as a simultaneous nitrogen and carbon source. Considered to be pathogenic, causing lung infections in humans. Source: sputum of humans. Type strain: E4796. A culture of this strain has been deposited in the American Type Culture Collection, Rockville,

TABLE 2. Characters useful for differentiating *M. shimoidei* from *M. malmoense*, *M. haemophilum*, and *M. ulcerans*

Character	<i>M.</i> <i>shim-</i> <i>oidei</i> (n = 4) ^a	<i>M.</i> <i>ulce-</i> <i>rans</i> (n = 3)	<i>M.</i> <i>malmo-</i> <i>ense</i> (n = 15)	<i>M.</i> <i>haemoph-</i> <i>ilum</i> (n = 1)
Rough colonies	100 ^b	0	0	—
Growth at 28°C	25	100	100	+
Growth at 45°C	100	0	0	—
Resistance to rifampin (25 µg/ml) ^c	100	0	100	+
Resistance to ethambutol (5 µg/ml) ^c	0	0	93	—
Resistance to 0.2% <i>p</i> -aminosalicylate ^c	100	0	100	+
Resistance to NH ₂ OH·HCl (0.125 mg/ml) ^c	25	67	100	+
Resistance to NH ₂ OH·HCl (0.25 mg/ml) ^c	0	0	100	+
Resistance to NH ₂ OH·HCl (0.5 mg/ml) ^c	0	0	100	—
Resistance to sodium salicylate (0.5 mg/ml) ^c	100	33	100	+
Resistance to <i>p</i> -nitrobenzoic acid (0.5 mg/ml) ^c	100	33	100	+
Tween hydrolysis after 14 days	100	0	93	—
Urease	0	0	60	—
Nicotinamidase	100	0	60	—
Pyrazinamidase	100	0	60	—
Acid phosphatase (3 h)	100	0	0	—
Catalase (semiquantitative) (foam > 45 mm)	0	0	93	—
Arylsulfatase after 14 days	0	0	100	+

^a The four strains of *M. shimoidei* are those isolated from a single patient.^b Values are percentages of strains which gave a positive reaction.^c Tested in Ogawa egg medium.

Maryland, under the number ATCC 27962. The description of the type strain is the same as that given above for the species.

REPRINT REQUESTS

Address reprint requests to: Dr. Michio Tsukamura, The National Chubu Hospital, Obu, Aichi, Japan 474.

LITERATURE CITED

- Jenkins, P. A., and M. Tsukamura. 1979. Infections with *Mycobacterium malmoense* in England and Wales. *Tubercle* 60:71–76.
- Liston, J., W. Wiebe, and R. R. Colwell. 1963. Quantitative approach to the study of bacterial species. *J. Bacteriol.* 85:1061–1070.
- Schröder, K. H., and I. Juhlin. 1977. *Mycobacterium malmoense* sp. nov. *Int. J. Syst. Bacteriol.* 27:241–246.
- Skerman, V. B. D., V. McGowan, and P. H. A. Sneath (ed.). 1980. Approved lists of bacterial names. *Int. J. Syst. Bacteriol.* 30:225–420.
- Sompolinsky, D., A. Lagziel, D. Naveh, and T. Yankilevitz. 1978. *Mycobacterium haemophilum* sp. nov., a new pathogen of humans. *Int. J. Syst. Bacteriol.* 28:67–75.
- Tsukamura, M. 1976. Numerical classification of slowly growing mycobacteria. *Int. J. Syst. Bacteriol.* 26:409–420.
- Tsukamura, M. 1976. An approach to numerical identification of bacterial species. *J. Gen. Microbiol.* 95:207–212.
- Tsukamura, M., H. Shimoide, and W. B. Schaefer. 1975. A possible pathogen of Group III mycobacteria. *J. Gen. Microbiol.* 88:377–380.
- Wayne, L. G., R. C. Good, M. I. Krichevsky, Z. Blacklock, S. D. Chaparas, D. Dawson, S. Froman, W. Gross, J. Hawkins, P. A. Jenkins, I. Juhlin, W. Käppler, H. H. Kleeberg, I. Krasnow, M. J. Lefford, E. Mankiewicz, C. McDermont, G. Meissner, E. E. Nel, S. R. Pattyn, F. Portaels, P. A. Richards, S. Rüsch, K. H. Schröder, I. Szabo, M. Tsukamura, and B. Vergmann. 1981. First report of the cooperative open-ended study of slowly growing mycobacteria (International Working Group on Mycobacterial Taxonomy). *Int. J. Syst. Bacteriol.* 31:1–20.