A Possible New Pathogen of Group III Mycobacteria

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INTRODUCTION

In the course of a co-operative study by eleven Japanese national chest hospitals on mycobacterioses due to mycobacteria other than *Mycobacterium tuberculosis*, a 56-year-old man was found to excrete a new group III mycobacterium. The organism was isolated on salicylate medium (Tsukamura, 1962) and identified as a new organism in 1968 in the research laboratory of the Chubu Chest Hospital. The man was a patient at the Tokyo Chest Hospital and had tuberculosis-like cavitory lesions in his lungs. Similar organisms were isolated twice in 1969 and thereafter were not evident until 1973. In 1973, the same organism was isolated from sputum by the use of a *p*-nitrobenzoic acid medium (Tsukamura & Tsukamura, 1964) in July and September, and the patient died that same year of the lung disease. *M. tuberculosis* was not isolated throughout the period of observation.

METHODS

A total of seven strains isolated between 1968 to 1973 were studied. Identification was carried out as described previously (Tsukamura, 1967*a*, 1969). Additional tests included lipid analysis using the method described by Marks, Jenkins & Schaefer (1971), and sero-agglutination studies with the method described by Schaefer (1965).

RESULTS AND DISCUSSION

All seven strains showed similar biological and biochemical characters.

Morphology. Gram-positive, acid-fast rods (0.5 to 1.0 by 2 to 3 μ m). Clumping or cord-like masses were not observed.

Biological characteristics. The organisms grew very slowly. Scant growth occurred on Ogawa egg medium and Löwenstein–Jensen medium after incubation at 37 °C for 3 weeks but egg media containing Tween 80 (0.5 %, w/v) gave good growth. The organisms were non-photochromogenic and grew as rough colonies on egg media. Growth occurred at 28, 37 and 45 °C but not at 52 °C.

Biochemical characteristics. The organisms did not grow on Sauton's agar containing 0.2 % (w/v) picric acid (the pH was adjusted to 7.0) or 0.1 % (w/v) NaNO₂. They did not utilize glucose, fructose, mannose, galactose, arabinose, xylose, rhamnose, trehalose,

Table 1. *Comparison of Mycobacterium shimoidei with known species of group III mycobacteria*

The symbols + and/or - give the properties of 'hypothetical median organisms' according to Liston, Wiebe & Colwell (1963); R, rough colonies; S, smooth colonies. The following strains were used for preparation of the hypothetical median organisms: 26 strains of *M. avium*; 76 strains of *M. intracellulare*; 6 strains of *M. xenopi*; 94 strains of *M. nonchromogenicum*; 15 strains of *M. terrae*; 21 strains of *M. triviale*; 26 strains of *M. novum*; 8 strains of *M. gastri*; 7 strains of present isolate. *Mycobacterium nonchromogenicum*, *M. terrae* (*M. novum*) and *M. triviale* showed similar characters in respect to the above and are presented as *M. nonchromogenicum* complex.

Character*	<i>M. avium</i>	<i>M. intracellulare</i>	<i>M. xenopi</i>	Present isolate (<i>M. shimoidei</i>)	<i>M. nonchromogenicum</i> complex	<i>M. gastri</i>
Colony morphology	S	S	S	R	S†	S
Two week-arylsulphatase (A)	-	+	+	-	+	+
α -Esterase (B)	+	+	+	+	-	-
Catalase (foam > 45 mm) (C)	-	-	-	-	+	+
Tween hydrolysis (14 days) (D)	-	-	-	trace	+	+
Resistance to 5 μ g ethambutol/ml (E)	+	+	+	-	-	-
Resistance to NH ₂ OH.HCl (F)						
0.25 mg/ml	+	+	-	-	+	-
0.5 mg/ml	+	+	-	-	+	-
Glucose as C source (glutamate-N) (G)	-	+	-	-	-	+
Growth at 28 °C	+	+	-	+	+	+
Growth at 45 °C	+	+	+	+	-	-
Urease (H)	-	-	-	-	-	+

* (A) Kubica & Beam (1961); (B) Kppler (1965); (C) Kubica *et al.* (1966); (D) Wayne, Doubek & Russell (1964); (E) Mizuno, Toyama & Tsukamura (1966), Tsukamura (1969); (F) Tsukamura (1965*b*); (G) Tsukamura & Tsukamura (1967); (H) Bnicke (1962).

† *Mycobacterium triviale* shows rough colonies (R) rather than smooth colonies (S).

‡ Several strains of *M. intracellulare* do not grow at 45 °C.

inositol, mannitol, sorbitol, ethanol, *n*-propanol, *n*-butanol, iso-butanol, acetate, citrate, succinate, malate, pyruvate, benzoate, fumarate, malonate, and propylene glycol as sole sources of carbon in the presence of ammoniacal nitrogen. Glucose, fructose, acetate, succinate, malate, pyruvate and fumarate were not utilized as sources of carbon in the presence of glutamate-nitrogen. Glutamate was not utilized as a simultaneous source of carbon and nitrogen. They grew on Ogawa egg medium containing 0.5 mg *p*-nitrobenzoic acid/ml, 10 μ g thiophene-2-carboxylic acid hydrazide/ml or 0.2 % (w/v) sodium *p*-aminosalicylate. They grew on Ogawa egg medium containing 0.125 mg NH₂OH.HCl/ml but not on this medium plus 0.25 mg NH₂OH.HCl/ml or 5 μ g ethambutol/ml. The organisms were niacin-negative, two week-arylsulphatase activity-negative and α - and β -esterase-positive. Nicotinamidase and pyrazinamidase activities were variable; other amidase activities (Bnicke, 1962) were not shown. Nitrate was not reduced to nitrite.

Susceptibility to antituberculous agents. Strains 4164, 4794, 4795, 4796, 7367 and 7380 were all resistant to (μ g/ml): streptomycin (20), isoniazid (0.1), *p*-aminosalicylate (1.0), ethionamide (40) and rifampicin (25); and were susceptible to (μ g/ml): ethambutol (5), kanamycin (100) and capreomycin (100).

Lipid analysis. Analysis of strains 4796, 7367 and 7380 for lipids did not reveal a specific lipid.

Seroagglutination. An antiserum against strain 4796, prepared according to the method of Schaefer (1965), was completely absorbed by strains 7367 and 7380, indicating serological

identity of the three strains. No other strain of group II or group III was agglutinated by this serum.

Slow growth, formation of non-photochromogenic colonies, negative nitrate reduction, and inability to grow on Sauton's agar containing 0.2 % picric acid and 0.1 % NaNO₂, are characters that place the test organisms in Runyon's (1959, 1965) group III mycobacteria (Tsukamura, 1969). The organisms were compared with known species of group III, *Mycobacterium avium* Chester (1901), *M. intracellulare* Runyon (1967), *M. xenopi* Schwabacher (1959), *M. nonchromogenicum* Tsukamura (1965*a*), *M. terrae* Wayne (1966), *M. triviale* Kubica *et al.* (1970), *M. novum* Tsukamura (1967*b*), (a probable synonym of *M. terrae* Wayne), and *M. gastri* Wayne (1966) (Table 1). The test organisms were differentiated from other known species by more than five characteristics. They are believed to constitute a new species with characters intermediate between the *M. avium*/*M. intracellulare* complex and the *M. nonchromogenicum* complex. The name *Mycobacterium shimoidei* Tsukamura sp. nov. is proposed for the new species (type strain No. 4796, ATCC27962). The specific epithet was taken from the name of the second author who first isolated the strains.

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