Mycobacterium parafortuitum: a New Species

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(Received 13 May 1965)

SUMMARY

A new species *Mycobacterium parafortuitum* is described. A number of strains of the genus *Mycobacterium* isolated from soil resembled *M. fortuitum* in their rapid growth, in their pattern of utilization of organic acids and in their pattern of amidase tests. However, these strains differed from *M. fortuitum* in the following points: they showed negative 3-day arylsulphatase test, they showed negative salicylate degradation and PAS degradation, they did not utilize nitrite as sole nitrogen source, and they formed acid from pentoses, arabinose, xylose, inositol and mannitol. This group of strains has been named as *M. parafortuitum* sp.nov.

INTRODUCTION

In the course of a study on 'atypical' mycobacteria isolated in Japan, the author isolated many acid-fast bacilli from the soil for comparison with the 'atypical' mycobacteria isolated from humans. Organisms isolated from soil, after treatment of soil samples with 4% (w/v) NaOH and 4% (v/v) H₂SO₄, were studied. Strains which were considered to be mycobacteria, on morphological grounds, were then classified according to their amidase pattern (Boenicke, 1962). Strains which showed the pattern characteristic for *Mycobacterium fortuitum* were then tested for 3-day arylsulphatase activity (Wayne, 1961), nitrite utilization (Tsukamura & Tsukamura, 1965), and salicylate and *p*-aminosalicylate (PAS) degradation (Tsukamura, 1965). A group of strains showing positive reactions in three or four of these tests was classified as *M. fortuitum*. However, several strains which showed negative reactions in all of these tests were also found. These strains are considered to be a new species and their characters are considered in this paper.

METHODS

Growth rate and temperature response were observed on Löwenstein-Jensen medium.

Amidase tests were carried out according to the method of Boenicke (1962).

Three-day arylsulphatase test was carried out according to the method of Wayne (1961), and *3-week arylsulphatase test* according to the method of Kubica & Vestal (1961).

Utilization of organic acid as sole carbon source was observed in the following medium: (NH₄)₂SO₄, 2.64 g.; KH₂PO₄, 0.5 g.; MgSO₄·7H₂O, 0.5 g.; purified agar (Wako Chemical Co., Osaka), 30.0 g.; distilled water, 1000 ml. This was adjusted to pH 7.0 by addition of 10% (w/v) KOH and the medium sterilized by autoclaving at 115° for 30 min. Organic acids (sodium salts) were sterilized at 100° for 15 min. and

added to medium at a concentration of 0.01 M. The medium was readjusted to pH 7.0 and sterilized at 100° for 10 min. Growth was observed after 2 weeks incubation at 37°.

Acid formation from sugars was tested in the following medium: NH_4Cl , 2.0 g.; KH_2PO_4 , 0.5 g.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g.; 0.2% (w/v) bromothymol blue in 0.02 M-NaOH, 20.0 ml.; purified agar, 30.0 g.; distilled water, 1000 ml. (pH 7.2). Sugar solutions were sterilized separately by heating at 100° for 5 min. and added to medium aseptically to a final concentration of 0.5% (w/v). Acid formation was observed after 2 weeks incubation at 37°.

Utilization of nitrogen compounds as sole nitrogen source (including sodium nitrite) was tested on the following medium: glycerol, 50.0 ml.; KH_2PO_4 , 0.5 g.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g.; sodium citrate, 2.0 g.; purified agar, 30.0 g.; distilled water, 950 ml. (pH 7.0). Nitrogen compounds were sterilized by heating at 100° for 5 min. or Seitz filtration (sodium nitrite) and added to medium aseptically to a final concentration of 0.02 M. Growth was observed after 2 weeks incubation at 37°.

Salicylate degradation was tested on Sauton agar medium containing 0.5 mg. and 1.0 mg. sodium salicylate/ml. Blackening of the medium after 7 days incubation at 37° was recognized as positive degradation.

PAS degradation was observed on Löwenstein-Jensen medium or Ogawa egg medium containing 1 mg. sodium *p*-aminosalicylate (PAS)/ml. Blackening of the medium after 7 days incubation at 37° was recorded as a positive reaction.

Pathogenicity to mice (CF1 strain) was tested by intravenous injection of the test strains in a dose of about 2 mg. (wet weight) per mouse. The viable numbers in the lungs and spleen were recorded as an index of pathogenicity. For the determination of the viable numbers in organs, three mice were killed every week and the lungs and spleens were homogenized (in a motor homogenizer) and mixed with 10 vol. saline containing 100 units/ml. penicillin G. The organ homogenates were diluted with saline to make 1/1–1/10⁴ dilutions: 0.02 ml. of each dilution was inoculated to Löwenstein-Jensen medium (five tubes for a dilution) and incubated at 37°. After 4 days incubation, the number of colonies was counted and viable numbers per organ calculated.

RESULTS

Characters of seven representative strains are shown in Table 1.

Morphology. Rods, 2–3 μ in length after cultivation for 3–4 days on Löwenstein-Jensen medium at 37°; acid-fast short bacillary to coccoid forms in old cultures. Non-motile. No cord formation in Dubos liquid medium without Tween. No mycelium was observed in slide culture even at early stage of incubation when acid-fast rods were present.

Löwenstein-Jensen medium. At 3–4 days growth was smooth, wet, thin, soft, creamy or pale yellow; at 10–14 days it became yellowish. A strain of group B (see below) was scotochromogenic. Strains 310 and 311 were mucoid.

Sauton agar medium. At 6–7 days growth was smooth, wet, soft, creamy or pale yellow. A strain of the group B was yellowish orange. In tables, non-photochromogenic strains were designated by 'n' and scotochromogenic (only one strain) by 's'.

Temperature relations. Some strains showed good growth at 28–45°, but no growth at 52°. These strains were named group A. Other strains showed little or

no growth at 45° (Table 1). This group was named group B. Neither group A nor group B strains survived 60° for 4 hr.

Salt tolerance. Growth variable in glycerol broth containing 5 % (w/v) NaCl.

Nitrate reduction: variable.

Table 1. *Physiological characteristics of Mycobacterium parafortuitum*

Property	Strain						
	Group A				Group B		
	N 310	N 311	N 304	N 314	N 305	N 307	S 312
Growth at							
52°	—	—	—	—	—	—	—
45°	+	+	+	+	±	—	—
37°	+	+	+	+	+	+	+
28°	+	+	+	+	+	+	+
Acid from							
Glucose	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+
Galactose	—	—	—	—	—	—	—
Arabinose	+	+	+	+	+	—	+
Xylose	+	+	+	+	+	+	+
Rhamnose	—	—	—	—	—	+	+
Trehalose	+	+	+	—	+	+	+
Lactose	—	—	—	—	—	—	—
Raffinose	—	—	—	—	—	—	—
Inositol	+	+	+	—	+	+	+
Mannitol	+	+	+	+	+	+	+
Sorbitol	—	—	—	—	—	—	—
Utilization of							
Acetate	+	+	+	+	+	+	+
Citrate	+	+	+	+	+	+	+
Succinate	+	+	—	+	+	+	+
Malate	+	+	+	+	+	+	+
Pyruvate	—	—	—	—	—	—	—
Benzoate	±	—	—	—	±	—	—
Utilization of							
Glycerol	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+
Sucrose	+	+	—	—	+	+	—
Propyleneglycol	+	+	+	—	+	+	+
Amidase for							
Acetamide	+	+	+	—	+	+	—
Benzamide	—	—	—	—	—	—	—
Urea	+	+	+	+	+	+	+
Isonicotinamide	—	—	—	—	—	—	—
Nicotinamide	+	+	+	+	+	+	+
Pyrazinamide	+	+	+	+	+	+	+
Salicylamide	—	—	—	—	—	—	—
Allantoin	+	+	+	—	+	+	—
Succinamide	—	—	—	—	—	—	—
Malonamide	—	—	—	—	—	—	—
NaNO ₃ reduction	—	+	—	+	+	+	+
3-day arylsulphatase test	—	—	—	—	—	—	—
3-week arylsulphatase test	+	+	+	+	+	+	+
Salicylate degradation	—	—	—	—	—	—	—
PAS degradation	—	—	—	—	—	—	—
NaNO ₂ utilization	—	—	—	±	—	—	—

Three-day arylsulphatase test: negative.

Three-week arylsulphatase test: positive.

Salicylate degradation: negative.

PAS degradation: negative.

Amidase tests. Amidase positive for acetamide, urea, nicotinamide, pyrazinamide, and allantoin. Amidase negative for benzamide, isonicotinamide, salicylamide, succinamide, and malonamide. Incubation: 15 hr. Temperature: 37°.

Utilization of organic acids. Acetate, citrate, succinate and malate were utilized as sole carbon sources. Pyruvate was not utilized. No growth or scanty growth on medium containing benzoate as sole carbon source.

Acid formation from sugars. Acid was formed from glucose, mannose, arabinose, xylose, trehalose, inositol, and mannitol. No acid from galactose, lactose, raffinose, and sorbitol; rhamnose variable. Good growth or moderate growth on media containing most sugars, but scanty growth on media containing lactose or raffinose.

Table 2. *Virulence to mice of strains of Mycobacterium parafortuitum*

Strain	Inoculum	Organ	Viable numbers per organ*			
			1 week	2 weeks	3 weeks	4 weeks
N310	1.3×10^8	Lungs	< 18	< 16	< 16	.
		Spleen	760	715	< 12	.
N311	2.0×10^8	Lungs	< 18	229	< 16	< 18
		Spleen	6300	3630	1350	24
N304	9.7×10^8	Lungs	< 23	< 20	< 18	.
		Spleen	440	< 30	< 22	.
N314	1.0×10^9	Lungs	< 10	< 15	< 17	.
		Spleen	< 17	124	< 25	.
N305	3.5×10^8	Lungs	< 15	< 26	< 27	< 20
		Spleen	6320	5330	545	< 24

* Average in three mice. Inoculation was made intravenously. At different intervals, the mice inoculated were sacrificed and viable numbers of bacteria were counted by dilution plating technique.

Utilization of nitrogen compounds as sole nitrogen source. L-Glutamate, L-leucine, L-glycine, L-serine, L-methionine, ammonium sulphate, ammonium chloride, sodium nitrate, nicotinamide, and urea were utilized. Isonicotinamide and pyrazinamide variable. Sodium nitrite not utilized.

Pathogenicity to mice. Intravenous injection of the strains at a dose of 2 mg. wet weight did not kill mice within 1 month. No macroscopic lesions were observed in the mice and intravenously injected bacilli were eliminated rapidly from mouse organs. Among the strains tested, N 311 and N 305 were eliminated more slowly than the other strains (Table 2).

DISCUSSION

In view of the results, the most useful characteristics for recognizing the strains described in this paper are: (1) rapid growth on egg media; (2) good growth at 45° for group A—this characteristic most easily differentiates this group from *M. fortuitum*; (3) negative 3-day arylsulphatase reaction; (4) inability to decompose salicylate or PAS; (5) failure to utilize sodium nitrite as sole nitrogen source within 1 week; (6) amidase positive for acetamide, urea, nicotinamide, pyrazinamide, and

allantoin; amidase negative for benzamide, isonicotinamide, salicylamide, succinamide, and malonamide after incubation at 37° for 15 hr; (7) ability to form acid from glucose, mannose, arabinose, xylose, trehalose, inositol, and mannitol; inability to form acid from galactose, raffinose, and sorbitol; (8) failure to utilize

Table 3. *Comparison of physiological characteristics of Mycobacterium fortuitum, M. parafortuitum, M. smegmatis, and M. rhodochrous*

Property	<i>M. fortuitum</i> *	<i>M. parafortuitum</i>	<i>M. smegmatis</i> *	<i>M. rhodochrous</i> *
Growth at				
at 52°	—	—	—	—
at 45°	—	Variable	+	Variable
at 37°	+	+	+	+
Acid from				
Glucose	+	+	+	+
Mannose	+	+	+	+
Galactose	Rare	—	+	Rare
Arabinose	Rare	+	+	—
Xylose	Rare	+	+	Rare
Rhamnose	—	Variable	+	Rare
Trehalose	+	+	+	+
Lactose	—	—	—	—
Raffinose	—	—	—	—
Inositol	Rare	+	+	Rare
Mannitol	Variable	+	+	+
Sorbitol	Rare	—	+	+
Utilization of benzoate	—	—	+	+
3-day arylsulphatase test	+	—	—	—
Salicylate degradation	+	—	—	.
PAS degradation	+	—	—	.
NaNO ₂ utilization	+	—	(+)	.

* Includes data taken from Gordon & Mihm (1959).

Table 4. *Comparison of physiological characteristics among Mycobacterium fortuitum, M. parafortuitum, and M. smegmatis*

Property	<i>M. fortuitum</i> *	<i>M. parafortuitum</i>	<i>M. smegmatis</i> *
Amidase for			
Acetamide	+	+	— or +
Benzamide	—	—	+
Urea	+	+	+
Isonicotinamide	—	—	+
Nicotinamide	+ or —	+	+
Pyrazinamide	+ or —	+	+
Salicylamide	—	—	— or +
Allantoin	+ or —	+	—
Succinamide	—	—	+
Malonamide	—	—	—

* Data taken from those of Boenicke (1962).

benzoate; (9) colonies slightly pigmented or non-pigmented; (10) rapid elimination from mouse body

By comparison of these data with the descriptions of Gordon & Mihm (1959), Gordon & Smith (1955), and Boenicke (1962), the strains described in this paper are differentiated from *Mycobacterium fortuitum*, *M. smegmatis*, *M. rhodochrous* and *M. phlei* (Tables 3, 4).

The most important reactions separating these strains from those of *Mycobacterium fortuitum* are: negative 3-day arylsulphatase test; negative salicylate and PAS degradation tests; failure to utilize nitrite as sole nitrogen source; acid production from arabinose, xylose, and inositol; growth at 45° (group A).

The most important reactions separating the strains from *Mycobacterium smegmatis* are: failure to form acid from galactose or sorbitol; failure to utilize benzoate as sole carbon source; negative reactions for benzamidase, isonicotinamidase, and succinamidase; positive reaction for allantoinase.

The most important reactions separating the strains from those of *Mycobacterium rhodochrous* are: acid production from arabinose, xylose and inositol; inability to form acid from sorbitol; inability to utilize benzoate as sole carbon source.

The most important reactions separating the strains from *Mycobacterium phlei* are: no growth at 52°; failure to survive 4 hr at 60°; acid production from inositol; inability to form acid from sorbitol; positive reaction for allantoinase.

By these comparisons, the strains described in this study were differentiated from the known species and considered to belong to a new species. The author proposes to name this group of strains *Mycobacterium parafortuitum* sp.nov.

Strains 310 and 311 have been deposited in the National Collection of Type Cultures, Colindale Avenue, London, N.W. 9.

Strain 310 is NCTC 10410.

Strain 311 is NCTC 10411.

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