Tsukamurella pulmonis sp. nov.

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Chemotaxonomic and 16S ribosomal DNA sequence analyses of an isolate from the sputum of a patient with a mycobacterial lung infection clearly delineated a new species of the genus *Tsukamurella*. This new species can be defined on the basis of genotypic and phenotypic data. The name *Tsukamurella pulmonis* sp. nov. is proposed for this organism; the type strain is IMMIB D-1321^T (= DSM 44142^T). This isolate shows 44.2 and 36.2% DNA relatedness to *Tsukamurella paurometabola* DSM 20162^T (T = type strain) and *Tsukamurella inchonensis* DSM 44067^T, respectively.

The taxonomic history of the genus *Tsukamurella*, particularly that of *Tsukamurella paurometabola*, was recently outlined in detail (36). In a numerical taxonomic study, Goodfellow et al. (7) proposed the name *Tsukamurella wratislaviensis* for a group of isolates that clustered to form a new center of variation most closely associated with *T. paurometabola*. These strains had chemical, enzymic, nutritional, and tolerance properties consistent with their assignment to the genus *Tsukamurella* and formed a DNA homology group that also corresponded to the group formed by *T. paurometabola*. Recently, Yassin et al. (36) described *Tsukamurella inchonensis*. This species has chemotaxonomic and phylogenetic properties consistent with its assignment to the genus *Tsukamurella*. A lipid analysis of *T. inchonensis* and *T. paurometabola* showed that both of these species have two mycolates that correspond to α - and α' -mycolates which on pyrolysis produce C_{20:1}, C_{20:0}, C_{22:1}, and C_{22:0} fatty acids as main cleavage products (some strains of *T. inchonensis* produce only C_{22:0}). The fatty acid profile comprises saturated, unsaturated, and 10-methyl branched fatty acids. Unsaturated MK-8, MK-9, and MK-10 menaquinones are present, and MK-9 is the predominant component. The 16S ribosomal DNA (rDNA) sequences of *T. inchonensis* and *T. paurometabola* show a high level of similarity (99.3%).

Tsukamura and Kawakami (29) first drew attention to the pathogenic potential of *Tsukamurella* strains in humans when they isolated *T. paurometabola* seven times from the sputa of a patient with tuberculosis-like disease. A case of fetal meningitis (22), a case of severe gangrenous tendosynovitis with multiple subcutaneous abscesses (28), and a case of bacteremia (24) have also been described. *T. inchonensis* was isolated four times from blood cultures of a patient who had ingested hydrochloric acid (36). This species was also isolated from necrotic material from a patient with lung carcinoma. The similarity of *Tsukamurella* strains to other more common pathogens expected in immunocompromised patients, such as *Rhodococcus*, *Nocardia*, *Gordona*, and *Mycobacterium* strains, may have resulted previously in inadequate diagnosis and thus in underestimating the incidence of *Tsukamurella* infections. Clinicians should therefore be aware of the pathogenic potential of *Tsuka-*

murella spp., especially when they are treating AIDS patients and similarly immunocompromised persons.

In this paper we describe a new *Tsukamurella* species which was isolated from the sputum of a 92-year-old woman suffering from pulmonary tuberculosis. *Mycobacterium tuberculosis* and *Mycobacterium fortuitum* were first isolated from the patient. During a second hospitalization (2 years later) *Mycobacterium gordonae* was recovered, and during a third hospitalization (1 year later) the species described in this paper was isolated and erroneously identified as *M. fortuitum*. However, attempts to amplify the genomic DNA of this organism with two different universal primers for mycobacteria, one having the sequence 5'-GAGTTTGATCCTGGCTCAGGA3' (primer 283 of E. Böttger) and the other having the sequence 5'-TGCA CACAGGCCACAAGGGA3' (Amplicor; Hoffman-LaRoche), were unsuccessful, and therefore the taxonomic status of the organism was questioned. Applying routine chemotaxonomic methods, we found that the organism appeared to be closely related to members of the genus *Tsukamurella*. This was then confirmed by further taxonomic and phylogenetic investigations.

MATERIALS AND METHODS

Bacterial strains. Strain IMMIB D-1321^T (T = type strain) was isolated from the sputum of a 92-year-old woman who suffered from pulmonary tuberculosis and possibly an additional mycobacterial infection. The source of *T. inchonensis* DSM 44067^T has been described previously (36). *T. paurometabola* DSM 20162^T was obtained from the German Collection of Microorganisms and Cell Cultures.

Isolation method. Strain IMMIB D-1321^T was isolated from the sputum after decontamination with *N*-acetyl-L-cysteine (14, 26). The specimen was concentrated by centrifugation at 3,000 × *g* for 20 min and was cultured on Loewenstein-Jensen medium.

Morphology and pigmentation. Strain IMMIB D-1321^T was grown on yeast extract-malt extract agar (International *Streptomyces* Project [ISP] medium 2), oatmeal agar (ISP medium 3), and inorganic salts-starch agar (ISP medium 4) as described by Shirling and Gottlieb (25) and was examined for pigmentation, production of aerial hyphae, and other morphological characteristics. Cultures were grown for 4 weeks and were observed weekly. Air-dried smears from brain heart infusion (BHI) agar cultures were stained by the Gram and Ziehl-Neelsen methods in order to determine the Gram reaction and acid fastness, respectively. The micromorphology of the organism was determined by using a culture grown at 37°C for 4 days on ISP medium 2. Electron micrographs of the preparation were taken with a Zeiss digital scanning electron microscope (model DSM 950).

Physiological characteristics. The ability of the organism to grow at various temperatures (24, 31, 37, and 45°C) was determined after 1 week of incubation on Loewenstein-Jensen medium slants and BHI agar. Peptone-yeast extract-iron agar (ISP medium 6) and tyrosine agar (ISP medium 7) as described by Shirling and Gottlieb (25) were used to determine melanoid pigment production. The following properties were determined as described previously: the niacin test was performed by using test strips (Difco Laboratories, Detroit, Mich.) (12, 32);

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FIG. 1. Phase-contrast micrographs of cells of strain IMMIB D-1321^T stained with lactophenol cotton blue, showing cells that cluster together either pole to pole or pole to side (A) to simulate a branched structure (B). A slide culture was grown on BHI agar for 6 h at $36 \pm 1^\circ\text{C}$.

nitrate reductase activity was determined by the method of Kubica and David (13); catalase activity was determined by the method of Kubica and Pool (15); arylsulfatase activity (after 3 days) was determined by the method of Kubica and Ridgon (16); β -glucosidase and β -galactosidase activities were determined by the method of Tsukamura (27); Tween 80 hydrolysis was determined by the method of Wayne et al. (31); growth on MacConkey agar was determined by the method of Pattyn and Portals (21); resistance to NaCl and *p*-nitrobenzoic acid was determined by the methods of Wayne et al. (31) and Tsukamura and Tsukamura (30); acetamidase, allantoinase, benzamidase, nicotinamidase, pyrazinamidase, succinamidase, and urease activities were determined by the method of Bönicke (2); and tests to determine resistance to streptomycin (4.0 and 8.0 μg), isoniazid (0.25 and 1.0 μg), ethambutol (8.0, 16.0, and 32.0 μg), rifampin (16.0 and 32.0 μg), *p*-aminosalicylic acid (16 and 32.0 μg), protion-amide (16.0 and 32.0 μg), capreomycin (16.0 and 32.0 μg), and cycloserine (16.0 and 32.0 μg) were performed by using Loewenstein-Jensen medium slants supplemented with these antibiotics (Biotest). Tests to determine decomposition of adenine, guanine, hypoxanthine, xanthine, tyrosine, elastin, keratin, and testosterone were performed by the method of Gordon and Smith (10); the test to determine esculin decomposition was performed by the method of Gordon (8); and casein and gelatin hydrolysis tests were performed by the method of Gordon and Mihm (9). The urea decomposition test was performed by using urea agar base (catalog no. CM 53; Oxoid) after 2.2% urea was added. In tests to determine utilization of substrates as carbon sources and as simultaneous carbon and nitrogen sources we

used the media described by Yassin et al. (36). Sensitivity to lysozyme was determined as described previously (34).

Cell chemistry. The strains which we studied were cultivated at 37°C in shake flasks (120 rpm) containing BHI (Difco) broth for 1 week. After we checked for purity at maximum growth, the organisms were killed with formaldehyde (1%, vol/vol), harvested by centrifugation, washed with distilled water, and freeze-dried. Analyses of whole-cell hydrolysates for amino acids and sugars were performed by the methods of Becker et al. (1) and Lechevalier (17). Acid methanolysis, alkaline methanolysis, conversion to *tert*-butyldimethylsilyl (TBDMS) ethers, one-dimensional thin-layer chromatography, two-dimensional thin-layer chromatography, and pyrolysis gas chromatography were performed as previously described by Yassin et al. (33). Menaquinones were extracted and purified by the method of Collins et al. (4) and were identified by using a Finnigan Mat 212 mass spectrometer. Phospholipids were extracted, purified, and identified as described previously (35).

DNA isolation and characterization. DNA was isolated by chromatography on hydroxyapatite by using the method of Cashion et al. (3). Guanine-plus-cytosine (G+C) contents were determined by high-performance liquid chromatography (20). DNA-DNA hybridization studies were carried out by using the thermal renaturation method as described previously (34).

16S rDNA isolation and sequence determination. Genomic DNA extraction and PCR-mediated amplification of 16S rDNA genes were performed as described previously (23). Purified PCR products were directly sequenced by using

FIG. 2. Scanning electron micrographs of strain IMMIB D-1321^T, showing long rod-shaped cells (A) that fragment into three parts (B), which separate and grow as independent cells (C). The culture was grown on ISP medium 2 for 4 days at $36 \pm 1^\circ\text{C}$.

a *Taq* DyeDeoxy terminator cycle sequencing kit (Applied Biosystems, Foster City, Calif.) according to the protocol provided by the manufacturer. An Applied Biosystems model 373A DNA sequencer was used to electrophorese the sequencing reaction mixtures.

The 16S rDNA sequence determined in this study was manually aligned with previously published sequences available from public databases. Evolutionary distances calculated by the method of Jukes and Cantor (11) were used to construct a phylogenetic tree by the least-squares method of De Soete (6).

TABLE 1. Differential physiological characteristics of strain IMMIB D-1321^T, *T. inchoensis* DSM 44067^T, and *T. paurometabola* DSM 20162^T

Characteristic	Strain IMMIB D-1321 ^T	<i>T. inchoensis</i> DSM 44067 ^T	<i>T. paurometabola</i> DSM 20162 ^T
Hydrolysis of:			
Adenine	—	—	—
Hypoxanthine	—	+	—
Tyrosine	—	—	—
Xanthine	—	—	—
Casein	—	—	—
Urea	+	+	+
Esculin	+	+	+
Gelatin	—	—	—
Elastin	—	—	—
Guanine	—	—	—
Keratin	—	—	—
Testosterone	—	—	—
Tween 80	+	+	+
Utilization of the following compounds as sole sources of carbon and energy			
Arabinose	—	—	—
Xylose	—	—	—
Glucose	+	+	+
Galactose	+	+	+
Rhamnose	—	—	—
Lactose	—	—	+
Maltose	—	+	—
Raffinose	—	—	+
Trehalose	+	+	+
Sucrose	+	+	+
Cellobiose	—	+	—
Melezitose	—	+	—
Adonitol	—	—	—
Erythritol	—	—	—
Inositol	—	+	+
Mannitol	+	+	—
D-Sorbitol	+	+	—
Acetate	+	+	+
Benzoate	—	—	—
Citrate	—	+	—
Gluconate	+	+	+
Lactate	—	—	—
<i>m</i> -Hydroxybenzoate	—	—	—
<i>p</i> -Hydroxybenzoate	—	—	—
Adipate	—	—	—
Isoamyl alcohol	+	+	—
2,3-Butandiol	+	+	+
1,2-Propandiol	+	+	—
Paraffin	+	+	+
Utilization of the following compounds as sole sources of carbon and nitrogen			
Acetamide	+	+	—
Alanine	+	+	+
Gelatin	—	—	—
Proline	+	+	+
Serine	—	+	—
Growth at:			
24°C	+	+	+
31°C	+	+	+
37°C	+	+	+
45°C	—	+	—
Growth on MacConkey agar without crystal violet	+	+	+
Niacin production	—	—	—
Enzymatic activities			
Acetamidase	+	+	+

TABLE 1—Continued

Characteristic	Strain IMMIB D-1321 ^T	<i>T. inchoensis</i> DSM 44067 ^T	<i>T. paurometabola</i> DSM 20162 ^T
Allantoinase	+	+	+
Arylsulfatase (3 days)	—	—	—
Benzamidase	—	—	ND ^a
Catalase	+	+	+
β-Glucosidase	+	+	+
β-Galactosidase	+	+	+
Nicotinamidase	+	+	+
Nitrate reductase	—	—	—
Pyrazinamidase	+	+	+
Succinamidase	—	—	ND
Urease	+	+	+
Phosphatase	+	+	ND
Growth in the presence of:			
Streptomycin	+	+	+
Isoniazid	+	+	+
Ethambutol	+	+	+
Rifampin	+	+	+
<i>p</i> -Aminosalicylic acid	+	+	+
Protionamide	+	+	+
Capreomycin	+	+	+
Cycloserine	+	+	+

^a ND, not determined.

Nucleotide sequence accession number. The 16S rDNA sequence of strain IMMIB D-1321^T is available from the EMBL data library under accession number X92981.

RESULTS AND DISCUSSION

Colonial morphology. Strain IMMIB D-1321^T grew well on ISP medium 2 and ISP medium 3; the colonies were cream colored and rough. On ISP medium 4, growth was weak and the colonies were leathery. No diffusible pigments were observed. At 24 to 37°C growth occurred within 1 to 2 days on Loewenstein-Jensen medium and on BHI agar. Dilute inocula on BHI agar yielded large, eugonic, cream-colored, rough colonies.

Micromorphology. The cells of strain IMMIB D-1321^T were rod shaped, gram positive, and slightly acid-alcohol fast. During growth the cells were primarily long rods (Fig. 2A) which stuck together, either pole to pole or pole to side (Fig. 1A), to give an arrangement that looked like branched hyphae (Fig. 1B). In a later stage of growth these rods appeared to fragment into three parts (Fig. 2B) which separated (Fig. 2C) and started growing again. Spores, capsules, true branching, and aerial hyphae were not observed.

Physiological characteristics. The physiological properties of strain IMMIB D-1321^T are shown in Table 1. This strain had acetamidase, allantoinase, catalase, β-glucosidase, β-galactosidase, nicotinamidase, pyrazinamidase, urease, and phosphatase activities; arylsulfatase, benzamidase, nitrate reductase, and succinamidase activities were not observed. Strain IMMIB D-1321^T did not produce niacin but tolerated 5% NaCl and *p*-nitrobenzoic acid. This strain grew in the presence of the antituberculous drugs streptomycin, isoniazid, ethambutol, rifampin, *p*-aminosalicylic acid, protionamide, capreomycin, and cycloserine.

Lipid analysis. The results of one-dimensional thin-layer chromatography and two-dimensional thin-layer chromatography of whole-cell acid and alkaline methanolsates of strain IMMIB D-1321^T are shown in Fig. 3. Two-dimensional thin-layer chromatography resulted in more precise resolution of

Continued

FIG. 3. (a and c) One-dimensional thin-layer chromatography and two-dimensional thin-layer chromatography of whole-cell alkaline (a) and acid (c) methanolysates of strain IMMIB D-1321^T. Triple development in the first direction was with petroleum ether (bp 60 to 80°C)-acetone (95: 5, vol/vol), and a single development in the second direction was with toluene-acetone (97: 3, vol/vol). Abbreviations: α, α-mycolate; α', α'-mycolate; F, nonhydroxylated fatty acid methyl esters. (b) TBDMS ethers of alkaline methanolysates. A single development in petroleum ether (bp 60 to 80°C)-toluene (70:30, vol/vol) was used. α and α' refer to TBDMS ethers of the different types of mycolic acid methyl esters.

the components into two mycolate spots in addition to the nonhydroxylated fatty acid spot (spot F). One-dimensional thin-layer chromatography of the TBDMS ethers of the alkaline methanolysates resulted in identification of the two mycolate spots as α- and α'-mycolates by their R_f values (Fig. 3).

Pyrolysis gas chromatography of the pure α-mycolates that were isolated by preparative thin-layer chromatography from strain IMMIB D-1321^T released C_{20:0}, C_{20:1}, C_{22:0}, and C_{22:1} fatty acid methyl esters as main pyrolytic cleavage products. Gas chromatographic analyses of the nonhydroxylated fatty acid methyl esters revealed the presence of hexadecanoate, octadecanoate, and tuberculostearic acid (10-methyl octadecanoate) as major fatty acid methyl esters. No 2-methyl branched-chain fatty acids were observed.

A polar lipid analysis showed that the phospholipid type of strain IMMIB D-1321^T is type PII sensu Lechevalier et al. (18); i.e., phosphatidylethanolamine is the characteristic phospholipid. Other phospholipids, including phosphatidylinositol, phosphatidylinositol mannoside, and diphosphatidylglycerol, were also detected. Strain IMMIB D-1321^T contains menaquinones MK-8, MK-9, and MK-10; MK-9 is the major component.

DNA characteristics. The G+C content of the DNA of strain IMMIB D-1321^T is 69.8 mol%. The levels of relatedness (binding rates) between strain IMMIB D-1321^T DNA and the DNAs of *T. paurometabola* DSM 20162^T and *T. inchonensis* DSM 44067^T are 44.2 and 36.3%, respectively (as determined by the optical method).

The almost complete 16S rDNA sequence of strain IMMIB D-1321^T was determined. A phylogenetic analysis showed that this strain is most closely related to the mycolic acid-containing actinomycetes and, more specifically, to the genus *Tsukamurella* (Fig. 4). The 16S rDNA sequence of strain IMMIB D-1321^T is very similar to the 16S rDNA sequences of *T. paurometabola* DSM 20162^T and *T. inchonensis* DSM 44067^T (levels of similarity, 99.3 and 99.4%, respectively) (Table 2). However, 16S rDNA sequence data showed that *T. wratislaviensis* falls outside the genus *Tsukamurella* and is a *Rhodococcus* species (unpublished data).

The physiological and chemotaxonomic characteristics of isolate IMMIB D-1321^T are compared with *T. inchonensis* DSM 44067^T and *T. paurometabola* DSM 20162^T characteris-

tics in Tables 1 and 3, respectively. Chemotaxonomically, strain IMMIB D-1321^T, *T. inchonensis* DSM 44067^T, and *T. paurometabola* DSM 20162^T are similar (Table 3). All three strains contain galactose and arabinose as characteristic whole-cell sugars in addition to *meso*-diaminopimelic acid as a wall diamino acid (i.e., they are wall chemotype IV organisms); all contain two mycolic acids corresponding to α- and α'-mycolates which on pyrolysis release C_{20:1}, C_{20:0}, C_{22:1}, and C_{22:0} fatty acids as mycolic acid cleavage products; the fatty acid profiles of all three consist of saturated, unsaturated, and 10-methyl branched fatty acids; and all contain unsaturated MK-8, MK-9, and MK-10 menaquinones, with MK-9 as the major

FIG. 4. Phylogenetic dendrogram showing the position of strain IMMIB D-1321^T within the mycolic acid-containing group. Scale bar = 5 nucleotide substitutions per 100 nucleotides. The sequences used in the analysis are the sequences of the type strains of the species included. Abbreviations: N., *Nocardia*; R., *Rhodococcus*; G., *Gordona*; T., *Tsukamurella*; M., *Mycobacterium*; D., *Dietzia*; C., *Corynebacterium*.

TABLE 2. Levels of 16S rDNA similarity between *T. pulmonis* and related taxa

Organism	% 16S rDNA similarity																
	<i>Tsukamurella pulmonis</i>	<i>Tsukamurella inchonensis</i>	<i>Tsukamurella paurometabola</i>	<i>Gordona bronchialis</i>	<i>Gordona rubropertincta</i>	<i>Gordona sputi</i>	<i>Gordona amarae</i>	<i>Rhodococcus rhodochrous</i>	<i>Rhodococcus globerulus</i>	<i>Rhodococcus equi</i>	<i>Nocardia asteroides</i>	<i>Nocardia otitidiscavarium</i>	<i>Mycobacterium aichiense</i>	<i>Mycobacterium tuberculosis</i>	<i>Mycobacterium terrae</i>	<i>D. maris</i>	<i>Corynebacterium xerosis</i>
<i>Tsukamurella inchonensis</i>	99.4																
<i>Tsukamurella paurometabola</i>	99.3	99.3															
<i>Gordona bronchialis</i>	93.2	93.3	93.4														
<i>Gordona rubropertincta</i>	93.1	93.1	93.3	98.5													
<i>Gordona sputi</i>	93.0	93.2	93.4	97.4	96.9												
<i>Gordona amarae</i>	94.1	94.4	94.6	97.1	97.1	96.4											
<i>Rhodococcus rhodochrous</i>	94.1	94.4	94.5	94.4	94.4	93.9	94.1										
<i>Rhodococcus globerulus</i>	94.8	94.7	94.8	93.8	93.4	92.7	93.4	95.1									
<i>Rhodococcus equi</i>	94.8	94.7	94.9	94.4	94.3	93.2	94.2	96.2	96.5								
<i>Nocardia asteroides</i>	94.4	94.6	94.7	93.5	93.4	92.7	94.0	94.0	95.2	96.3							
<i>Nocardia otitidiscavarium</i>	94.4	94.7	94.6	94.8	94.6	93.6	94.5	95.2	95.9	97.3	98.0						
<i>Mycobacterium aichiense</i>	94.2	94.5	94.6	93.7	93.2	93.3	94.0	93.9	94.0	93.7	94.1	94.1					
<i>Mycobacterium tuberculosis</i>	92.4	92.2	92.3	92.7	91.8	91.2	91.9	91.9	92.1	92.3	92.7	91.9	95.5				
<i>Mycobacterium terrae</i>	93.7	94.1	94.1	93.2	92.7	92.7	93.3	93.4	93.2	93.1	93.5	93.1	97.3	96.8			
<i>D. maris</i>	95.2	95.1	95.3	93.1	92.8	92.8	93.8	94.6	94.9	94.2	93.3	93.4	93.7	91.9	93.1		
<i>Corynebacterium xerosis</i>	92.2	92.3	92.3	92.2	91.4	91.1	91.2	92.4	91.9	91.9	90.7	91.5	91.7	92.2	92.2	93.4	
<i>Corynebacterium glutamicum</i>	90.4	90.4	90.5	90.0	89.9	89.4	89.4	91.4	90.7	90.7	89.1	89.8	89.6	89.1	89.5	91.2	93.5

component. These chemotaxonomic similarities are supported by the high levels of 16S rDNA similarity observed with isolate IMMIB D-1321^T, *T. inchoensis* DSM 44067^T, and *T. paurometabola* DSM 20162^T (Table 2).

In contrast to the chemotaxonomic similarities of isolate IMMIB D-1321^T, *T. inchoensis* DSM 44067^T and *T. paurometabola* DSM 20162^T, the results of our physiological tests (Table 1) revealed clear differences between strain IMMIB D-1321^T and the other two strains. Physiologically, isolate IMMIB D-1321^T differs from *T. inchoensis* DSM 44067^T by (i) its inability to hydrolyze hypoxanthin, (ii) its inability to utilize maltose, cellobiose, melzitose, inositol, and citrate as carbon sources, and (iii) its inability to grow at 45°C. Strain IMMIB D-1321^T differs from *T. paurometabola* DSM 20162^T by (i) its ability to utilize mannitol, sorbitol, isoamyl alcohol, and 1,2-propandiol as carbon sources and (ii) its ability to utilize acetamide as a simultaneous carbon and nitrogen source. These physiological differences are supported by the low levels of hybridization between strain IMMIB D-1321^T

DNA and the DNAs of *T. inchoensis* DSM 44067^T (36.3%) and *T. paurometabola* DSM 20162^T (44.2%).

Thus, we propose that strain IMMIB D-1321^T should be placed in a new species of the genus *Tsukamurella*. This strain has been deposited in the German Collection of Microorganisms and Cell Cultures as strain DSM 44142^T. The type strain was isolated from the sputum of a 92-year-old woman with cured pulmonary tuberculosis who later developed signs of pulmonary infection again. The patient recovered from the infection only after antibiotics that were active against the *Tsukamurella* strain in question had been administered. Strain IMMIB D-1321^T therefore appears to be pathogenic and should be placed in risk group 2 according to the German System. A description of this species is given below.

Description of *Tsukamurella pulmonis* sp. nov. *Tsukamurella pulmonis* (pul. mo' nis. L. gen. masc. n. *pulmonis*, of the lung, referring to the organ from which the bacterium was isolated). Cells are aerobic, gram-positive, and slightly acid-alcohol-fast bacilli. Most cells are long rods that, at a later stage of growth,

TABLE 3. Chemotaxonomic characteristics of strain IMMIB D-1321^T, *T. inchoensis* DSM 44067^T, and *T. paurometabola* DSM 20162^T

Strain	Wall diamino acid	Wall sugars	Wall chemo-type ^a	Phospholipid type ^b	Menaquinones ^c	Mycolic acid composition	Mycolic acid pyrolysis products	Fatty acids ^d
<i>T. pulmonis</i> IMMIB D-1321 ^T	meso-DAP ^e	Galactose, arabinose	IV	PII	MK-10, MK-9, MK-8	α, α'	C _{20:0} , C _{20:1} , C _{22:0} , C _{22:1}	S, U, T
<i>T. inchoensis</i> DSM 44067 ^T	meso-DAP	Galactose, arabinose	IV	ND ^f	MK-10, MK-9, MK-8	α, α'	C _{20:0} , C _{20:1} , C _{22:0} , C _{22:1}	S, U, T
<i>T. paurometabola</i> DSM 20162 ^T	meso-DAP	Galactose, arabinose	IV	ND	MK-9, MK-8, MK-7 ^g	α, α'	C _{20:0} , C _{20:1} , C _{22:0} , C _{22:1}	S, U, T

^a Wall chemotype as described by Lechevalier and Lechevalier (19).

^b Phospholipid type as described by Lechevalier et al. (18).

^c MK-10 is a menaquinone with 10 isoprene units.

^d S, straight chain; U, monounsaturated; T, tuberculostearic acid.

^e meso-DAP, meso-diaminopimelic acid.

^f ND, not determined.

^g Data from reference 5.

fragment into three parts which then separate and grow as independent rods. The cells do not form spores, capsules, or aerial hyphae. Visible growth from dilute inocula occurs within 2 days; colonies on Loewenstein-Jensen medium and BHI agar are eugonic, creamy colored, and rough. Growth occurs at 24, 31, and 37°C, but not at 45°C. The organism contains meso-diaminopimelic acid as a wall diamino acid in addition to galactose and arabinose in whole-cell hydrolysates (i.e., the cell wall chemotype is chemotype IV). It has a mycolate pattern of the α , α' type and a type PII phospholipid pattern with significant amounts of phosphatidylethanolamine. MK-9 is the major menaquinone, and minor amounts of MK-10 and MK-8 are also present. Melanoid pigments are not produced on either ISP medium 6 or ISP medium 7. Produces acetamidase, allantoinase, catalase, β -glucosidase, β -galactosidase, nicotinamidase, pyrazinamidase, urease, and phosphatase but not arylsulfatase (after 3 days), benzamidase, nitrate reductase, and succinamidase. Grows on MacConkey agar without crystal violet and tolerates 5% NaCl and *p*-nitrobenzoic acid. Niacin is not produced. Resistant to lysozyme. Glucose, galactose, trehalose, sucrose, mannitol, sorbitol, acetate, gluconate, isoamyl alcohol, 2,3-butandiol, 1,2-propandiol, and paraffin are utilized as sole carbon sources, but arabinose, xylose, rhamnose, lactose, maltose, raffinose, cellobiose, melezitose, adonitol, erythritol, inositol, benzoate, citrate, lactate, *m*-hydroxybenzoate, *p*-hydroxybenzoate, and adipate are not utilized. Acetamide, alanine, and proline are utilized as nitrogen and carbon sources, but gelatin and serine are not utilized. Urea, esculin, and Tween 80 are hydrolyzed, but adenine, hypoxanthine, tyrosine, xanthine, casein, gelatin, elastin, guanine, keratin, and testosterone are not hydrolyzed. Acid is produced from glucose, galactose, trehalose, sucrose, mannitol, sorbitol, mannose, and fructose, but not from arabinose, xylose, rhamnose, lactose, maltose, raffinose, cellobiose, melezitose, adonitol, inositol, and inulin. Resistant to streptomycin, isoniazid, ethambutol, rifampin, *p*-aminosalicylic acid, protionamide, capreomycin, and cycloserine. The G+C content is 69.8 mol%. The type strain of *T. pulmonis* is strain DSM 44142.

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