CURRENT MICROBIOLOGY, Vol. 3 (1980), pp. 255–257

Current Microbiology
An International Journal

Legionella micdadei species nova: Classification of a Third Species of *Legionella* Associated with Human Pneumonia

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Abstract. Deoxyribonucleic acid relatedness studies were used to characterize three *Legionella*-like organisms; TATLOCK, HEBA, and the Pittsburgh pneumonia agent. The results showed that these three strains belong in the same species, which is distinct from the previously described species *Legionella pneumophila* and *L. bozemanii*. *Legionella micdadei* species nova is proposed for this new species. The type strain of *L. micdadei* is TATLOCK (ATCC 33218).

In 1943, Tatlock recovered an organism from three of five guinea pigs inoculated intraperitoneally with defibrinated blood from one of several soldiers with "Fort Bragg fever". The organism was a poorly staining, Gram-negative rod that grew well in yolk sacs of fertile, 7-day-old hens' eggs, but would not grow on any artificial media. Tatlock could not relate this agent to "Fort Bragg fever" through convalescent-phase serum reactions, but published his findings to record the characteristics of the rickettsia-like organism [14]. Subsequently, this organism was designated TATLOCK.

In 1959, Bozeman recovered another rickettsia-like organism from one of three guinea pigs inoculated with blood from a patient with suspected pityriasis rosea. This agent was designated HEBA, and like TATLOCK, would not grow on artificial media and could not be related to the patient by complement fixation tests. TATLOCK and HEBA had identical growth and staining characteristics, and cross-immunity was demonstrated in convalescent guinea pigs challenged with viable organisms [1].

In 1979, Hébert grew both TATLOCK and HEBA on the charcoal yeast extract (CYE) agar developed by Feeley, Gorman, and Gibson [6] for the primary isolation of *Legionella pneumophila*. The two "new bacteria", TATLOCK and HEBA, were shown to have identical cultural, biochemical, and antigenic characteristics, the same cellular fatty acid composition, and identical antimicrobial susceptibility profiles [7]. Preliminary deoxyribonucleic acid (DNA) studies showed that TATLOCK and HEBA were highly related and probably belonged to the same species; they were not related to *L. pneumophila* at the species level [7].

In 1979, Pasculle isolated two strains of an organism in guinea pigs and embryonated hens' eggs inoculated with lung tissue from two renal-transplant recipients with acute purulent pneumonia [13]. This Gram-negative bacillus did not grow on artificial media and was designated the "Pittsburgh pneumonia agent" (PPA). After Pasculle furnished Hébert et al. with yolk material from infected embryonated eggs, the PPA was cultivated on CYE agar and characterized. PPA was essentially identical in phenotypic tests to the TATLOCK bacterium and highly related to it by DNA hybridization [8]. Myerowitz et al. [12] recently reported the isolation in embryonated eggs of a third strain of the PPA from an immunosuppressed patient with acute pneumonia.

The direct fluorescent-antibody (FA) conjugate for TATLOCK that was described in an earlier report [7] is being used on clinical specimens submitted to the Center for Disease Control for study. Lung tissue sections from several pneumonia patients in Virginia contained numerous fluorescent bacteria when tested with the TATLOCK conjugate (B. M. Thomason, E. P. Ewing, M. D. Hicklin, S. A. Harding, and G. R. Donowitz, submitted for publication). Other specimens reported positive with this conjugate include fresh pleural fluid and scrapings of Formalin-fixed human lung tissue (B. M. Thomason, unpublished). The TATLOCK bacterium represents a distinct species, and its clinical significance is rapidly

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0343-8651/80/0003-0255 $01.00

Table 1. DNA relatedness of TATLOCK, HEBA, and PPA to each other and to *Legionella* species.

Source of unlabeled DNA	Source of labeled DNA: TATLOCK RBR, 60°C[a]	TATLOCK % D[b]	TATLOCK RBR, 75°C	*L. pneumophila* Philadelphia 1 RBR, 60°C	*L. bozemanii* WIGA RBR, 60°C	*L. bozemanii* WIGA RBR, 75°C
TATLOCK	100	0.0	100	5	0	0
HEBA	100	0.0	98	5	0	0
PPA	90	0.0	77	NT[c]	NT	NT
L. pneumophila strains:						
Philadelphia 1	4		1	100	5	0
Pontiac 1	5		NT	91	13	6
Bellingham 1	4		NT	92	8	7
Bloomington 1	0		0	75	2	2
L. bozemanii strains:						
WIGA	0		1	8	100	100
MI-15	6		2	25	56	64

[a] RBR, relative binding ratio = (% heterologous DNA bound to hydroxyapatite)/(% homologous DNA bound to hydroxyapatite) × 100.

[b] % D, percent divergence = the decrease in thermal stability (in °C) of heterologous DNA duplexes compared to that of the homologous DNA duplexes. It can be expressed as % because each degree decrease in thermal stability is caused by approximately 1% unpaired bases in double-stranded DNA.

[c] Not tested in this study.

becoming recognized.

TATLOCK, HEBA, and PPA are bacteria phenotypically similar to, but yet genetically distinct from *L. pneumophila.* The DNA relatedness studies of these three strains are presented in this report. On the basis of these and previous data, we place the TATLOCK bacterium in the genus *Legionella* as a new species, *Legionella micdadei.*

Materials and Methods

The TATLOCK, HEBA, and PPA strains have been described [7,8]. The WIGA strain [1,7,9] and the WIGA-like strain, MI-15 [5] (GA-PH [15]), of *Legionella bozemanii* [4] have been described. The Philadelphia 1, Pontiac 1, Bellingham 1, and Bloomington 1 strains of *L. pneumophila* have been described [2,3]. Strains were harvested from CYE agar plates for DNA hybridization. Isolation and purification of labeled and unlabeled DNA and details of the hydroxyapatite procedure for DNA hybridization have been described [3].

Results and Discussion

Labeled DNA from TATLOCK, *Legionella pneumophila,* and *L. bozemanii* was allowed to reassociate with unlabeled DNA from TATLOCK, HEBA, PPA, and strains of *L. pneumophila* [2] and *L. bozemanii* [4]. The results (Table 1) indicate that TATLOCK, HEBA, and PPA belong to the same species and that they are a separate species from *L. pneumophila* and *L. bozemanii.* Labeled TATLOCK DNA was 90% or more related to unlabeled DNAs from HEBA and PPA in 60°C reactions. There was no evidence of divergence (unpaired bases) within the related nucleotide sequences, and relatedness remained high in 75°C reactions in which only closely related sequences can reassociate. Strains of a species usually are 70% or more related in 60°C reactions, contain less than 6% divergence within related sequences, and show 60% or more relatedness in 75°C reactions. By all of these criteria, TATLOCK, HEBA, and PPA belong to the same species.

TATLOCK was only minimally related to several strains of *L. pneumophila* and to the two known strains of *L. bozemanii.* These data were confirmed in reciprocal reactions in which labeled DNA from *L. pneumophila* strain Philadelphia 1 and *L. bozemanii* strain WIGA were 5% and 0%, respectively, related to unlabeled DNAs from TATLOCK and HEBA. Relatedness results vary considerably when the DNA preparations are labeled in vitro, as these were. We therefore do not stress the quantitative results; however, the qualitative interpretation of these data leaves no doubt that TATLOCK, HEBA, and PPA are not members of either *L. pneumophila* or *L. bozemanii.*

TATLOCK, HEBA, and PPA are phenotypically identical [7,8]. They were initially isolated on CYE agar after 3–4 days of incubation in a moist atmosphere at 35°C. After several transfers on CYE agar, all three strains grew on Feeley-Gorman agar [6] and caused browning of the agar. They did not grow on commonly used laboratory media, such as blood agar or nutrient agar. Colonies on CYE agar were a dull yellow under long-wavelength ultraviolet excitation. They are Gram-negative bacilli that are not acid fast with the Ziehl-Neelsen stain for mycobacteria. Each of these strains has a polar flagellum. Their biochemical reactions are identical to those of *L. pneumophila.* They do not produce urease, reduce nitrate to nitrite, or ferment carbohydrates, but they liquify gelatin, utilize starch, and produce catalase and oxidase. Unlike *L. pneumophila,* cultures of TATLOCK, HEBA, and PPA do not produce a β-lactamase.

A TATLOCK direct FA reagent had the same high titer with TATLOCK, HEBA, and PPA, but did not stain any of 220 other organisms tested, including *L. pneumophila* and *L. bozemanii.* The cellular fatty acid composition of these bacteria, like that of *L. pneumophila* and *L. bozemanii,* is unusual for Gram-negative bacteria because of the large amount of total branched-chain acids. The major acid in TATLOCK, HEBA, and PPA is a-15:0, and the second

Table 2. Phenotypic characteristics that differentiate the three defined species of *Legionella*.

Characteristics	*L. micdadei*	*L. pneumophila*	*L. bozemanii*
Growth on F-G and MH-IH[a]	Adapted	Primary	Adapted
Fluorescence on CYE agar[b]	Dull yellow	Dull yellow	Blue-white
Production of β-lactamase	−	+	±

[a] F-G, Feeley-Gorman Agar; MH-IH, supplemented Mueller-Hinton agar [6]. Primary, initial isolation and fresh isolates from charcoal yeast extract (CYE) agar; adapted, growth can be obtained only after several successive transfers on CYE agar.
[b] Under long-wavelength ultraviolet excitation.

most abundant acid is a-17:0 [7,8]. They contain only trace amounts of i-14:0 acid, and differ from other legionellae in that they contain small amounts of an a-17:1 acid. *L. bozemanii* also has a-15:0 as its major acid, but i-16:0 is its next most abundant acid; a-17:0 is its third major acid; it contains small amounts of i-14:0 acid, and lacks the a-17:1 acid [7,10]. The major acid in *L. pneumophila* is the i-16:0 [11].

The name *Legionella micdadei* species nova (pronounced mick-day'-dee) is proposed for the species represented by the strains TATLOCK, HEBA, and PPA. The name is in honor of Joseph E. McDade, who first isolated *L. pneumophila*. He has contributed much to our understanding of all three *Legionella* species. The type strain of *L. micdadei* is TATLOCK (ATCC 33218), isolated by Hugh Tatlock in 1943 [14].

The phenotypic characteristics of *L. micdadei* are quite similar to those of *L. pneumophila* and *L. bozemanii* [2,4,7,8]. Characteristics of differential value are listed in Table 2; however, for one to properly identify an unknown isolate to the species level, direct FA staining reactions and cellular fatty acid composition must be determined.

ACKNOWLEDGMENTS

We are indebted to many of our colleagues in the Bacteriology Division, Bureau of Laboratories, and the Bacterial Diseases Division, Bureau of Epidemiology, for their assistance during these studies. We thank J. C. Feeley, G. W. Gorman, J. E. McDade, R. M. McKinney, C. W. Moss, and R. E. Weaver for providing several of the strains.

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