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Legionella gormanii sp. nov.

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A new species of *Legionella* was isolated from soil collected from a creek bank. The name *Legionella gormanii* sp. nov. is proposed.

After the outbreak of Legionnaires disease in Philadelphia, the etiological agent, a bacterium, was isolated and named *Legionella pneumophila* (2). Since then at least six serogroups of *L. pneumophila* have been identified (5a, 12, 13). A number of *Legionella*-like organisms (LLO) also have been described (5, 7) and shown to be *Legionella* species separate from *L. pneumophila*. Names have now been proposed for most of these organisms.

The LLO strain WIGA was first isolated by Bozeman in guinea pigs and embryonated eggs and has been named *Legionella bozemanii*; a second strain, MI-15, was shown to be of the same species (1). Two additional LLO strains, NY-23 isolated from a cooling tower (5) and Tex-KL isolated from a postmortem lung specimen (10), represent another species for which the name *Legionella dumoffii* has been proposed (1). In 1979 an outbreak of pneumonia occurred among patients in Pittsburgh, Pa. The causative agent, at first called Pittsburgh pneumonia agent, is of the same species as LLO strains TATLOCK and HEBA, and the name *Legionella micdadei* has been proposed (8). Another name, *Legionella pittsburgensis*, has also been proposed (15).

An LLO isolate that is phenotypically similar to, but genetically distinct from, the four named species of *Legionella* has been reported and referred to as LS-13 (1, 5). It was isolated from wet soil collected from a creek bank during an investigation of Legionnaires disease caused by *L. pneumophila* at a country club in Atlanta (4). The method of isolation was similar to that previously described for isolating *L. pneumophila* from environmental samples (14). The procedure involved the injection of a suspension of the soil in distilled water into two guinea pigs. One of the two guinea pigs had a fever for 2 days and was killed and examined at that time. The second guinea pig did not have a fever but was killed and examined at 7 days after injection. Spleen tissue from each guinea pig was inoculated to Feeley-Gorman and charcoal yeast extract agars and injected into six embryonating eggs (6). Embryos in the two eggs inoculated with spleen tissue from the first guinea pig died between 3 and 12 days. The egg yolk sacs were harvested and cultured on Feeley-Gorman and charcoal yeast extract agar. Embryos in eggs inoculated with tissue from the second guinea pig did not die. *Legionella*-like growth resulted on only one plate—charcoal yeast extract agar inoculated with egg yolk sac. No growth was observed on any of the other plates inoculated with yolk sac or guinea pig spleen.

The phenotypic characteristics of LS-13 were compared with those of the four described *Legionella* species and found to be very similar to those of *L. dumoffii* and *L. bozemanii* in that LS-13 emitted a blue-white fluorescence on exposure to long-wavelength (366-μm) ultraviolet light and produced beta-lactamase (Table 1). LS-13 differed in that it did not grow on Feeley-Gorman agar and it had serologically distinctive somatic antigens.

Strain LS-13 was distinguishable by direct immunofluorescence from the other four species of *Legionella*. The LS-13 strain did not stain when reacted with hyperimmune fluorescein isothiocyanate-conjugated rabbit antisera prepared against all species of *Legionella*, including all serogroups of *L. pneumophila*. However, a direct immunofluorescence conjugate prepared with antiserum against strain LS-13 reacted 4+ with the LS-13 strain but was nonreactive with isolates of *L. dumoffii*, *L. bozemanii*, *L. micdadei*, and *L. pneumophila* (all serogroups) at routine test dilution.

Gas-liquid chromatographic analysis of the cellular fatty acids of strain LS-13 showed an abundance of branched-chain fatty acids similar to those of other legionellae. The cellular fatty acid profile of LS-13 was similar to those of *L. bozemanii* and *L. dumoffii* in that the major acid was a-15:0 rather than i-16:0 as in *L. pneumophila* (5, 7, 10). Unlike *L. micdadei*, LS-13

TABLE 1. *Phenotypic characteristics of LLO strain LS-13 compared with other species of Legionella*

Characteristic	LS-13	*L. pneumophila*	*L. bozemanii*	*L. dumoffii*	*L. micdadei*
Growth on:					
Charcoal yeast extract agar	+	+	+	+	+
Feeley-Gorman agar	−	+	+[a]	+[a]	+[a]
Blood agar	−	−	−	−	−
Fluorescence	Blue-white	Dull yellow	Blue-white	Blue-white	Dull yellow
Browning on Feeley-Gorman agar	NG[b]	+	+	+	−
Gram stain	−	−	−	−	−
Flagella	+	+	+	+	+
Oxidase	−	+	−	−	+
Catalase	+	+	+	+	+
Urease	−	−	−	−	−
Gelatinase	+	+	+	+	+
β-Lactamase	+	+[c]	+[d]	+[e]	−[d]
$NO_3 \rightarrow NO_2$	ND[f]	−[d]	−	−[e]	−
Starch utilization	ND	+	+[d]	+[e]	+[f]
Acid from carbohydrates	−	−	−	−	−

[a] Adapted for growth on this medium after passage on charcoal yeast extract agar.
[b] NG, No growth on Feeley-Gorman agar. LS-13 did grow and produce browning on media containing 1% yeast extract with 0.025% ferric pyrophosphate soluble, 0.04% L-cysteine HCl, 0.04% L-tyrosine, and 1.7% agar.
[c] From C. Thornsberry and L. A. Kirven (16).
[d] From G. A. Hebert et al. (7).
[e] From K. R. Lewallen et al. (10).
[f] ND, Not determined; does not grow in medium.

TABLE 2. *DNA relatedness of LS-13 to legionellae*

Source of unlabeled DNA	Relative binding ratio[a] (%)									
	LS-13		*L. pneumophila* Philadelphia 1		*L. bozemanii* WIGA		*L. dumoffii* NY-23		*L. micdadei* TATLOCK	
	60°C	75°C	60°C	75°C	60°C	75°C	60°C	75°C	60°C	75°C
LS-13	100	100	20	NT	22	0	25	6	6	5
L. pneumophila Philadelphia 1	1	0	100	100	NT	NT	6	3	4	1
L. pneumophila Pontiac 1	NT	NT	91	90	13	6	NT	NT	5	NT
L. bozemanii WIGA	20	0	14	NT	100	100	22	4	0	1
L. bozemanii MI-15	33	4	25	NT	56	64	20	5	6	2
L. dumoffii NY-23	28	0	16	6	15	1	100	100	3	5
L. dumoffii TEX-KL	19	2	13	8	20	4	90	88	1	3
L. micdadei TATLOCK	8	0	5	NT	0	0	0	1	100	100
L. micdadei HEBA	NT	NT	5	NT	0	0	9	NT	100	98

[a] Relative binding ratio, (percent heterologous DNA bound to hydroxyapatite/percent homologous DNA bound to hydroxyapatite) × 100. The percent of DNA bound to hydroxyapatite in homologous reactions was arbitrarily deemed to be 100%. All reactions were repeated at least twice. Control reactions with only labeled DNA usually showed 3 to 10% binding to hydroxyapatite. These control values were subtracted from all reactions before normalization. Unlabeled DNA from *Proteus mirabilis* strain PM-1, which has a guanine plus cytosine content similar to that of legionellae, was included as a control for nonspecific reactions with most of the labeled DNA preparations. The relatedness values of PM-1 DNA to labeled *Legionella* DNAs, after subtraction of the label only control, were 0 to 6%. The techniques used in DNA hybridization were discussed previously (2, 3). Some of the data from labeled DNAs other than LS-13 were previously published (1) and are presented here for comparison of reciprocal reactions. NT, not tested in this study.

did not contain a-17:1 (5, 9, 15).

Relatedness among strain LS-13 and species of *Legionella* was determined by deoxyribonucleic acid (DNA) hybridization. Sheared, denatured, in vitro ^{3}H-labeled DNA from *L. pneumophila* strain Philadelphia 1, *L. bozemanii* strain WIGA, *L. micdadei* strain TATLOCK, and *L. dumoffii* strain NY-23 was reacted with similarly treated unlabeled DNA from a series of *Legionella* strains under conditions that allow DNA reassociation (Table 2). Regardless of whether LS-13 DNA or DNA from another *Legionella* species was labeled, LS-13 was ≤33% related to other legionellae. This level of relat-

edness is far below the 70% or more seen between strains of the same species. Thus, LS-13 does not belong to any of the previously described *Legionella* species.

We have collected evidence of infection with strain LS-13 in both humans and experimental animals. During a retrospective medical record review of patients in Connecticut who had a clinical syndrome compatible with Legionnaires disease, a formalinized autopsy lung specimen from a 69-year-old man who died with pneumonia in 1978 revealed typical cells with a 4+ fluorescence to a conjugate prepared with antiserum to LS-13. There were no detectable reactions to conjugates against *L. pneumophila* serogroups 1 to 6, *L. bozemanii* (WIGA), *L. micdadei* (TATLOCK), or *L. dumoffii* (NY-23). Paired sera from three patients analyzed at the Bacterial Immunology Branch at the Center for Disease Control by the indirect fluorescent antibody test for *Legionella* have shown a ≥4-fold rise to LS-13, but were negative for the other *Legionella* species (H. Wilkinson, personal communications). Evidence of infection in guinea pigs is that the guinea pigs yielding the isolate of LS-13 had a disease resulting in fever for 2 days before autopsy.

These data indicate that LS-13 is closely related to the other four species of *Legionella* on the basis of growth characteristics, biochemical reactions, and gas-liquid chromatography profile. It shows DNA relatedness to other legionellae but is distinct from all named species. Despite the fact that the only strain isolated thus far was from the environment, it is likely that this organism is responsible for human legionellosis.

We therefore propose that strain LS-13 be designated a new species in the genus *Legionella* and that it be given the name *Legionella gormanii* (pronounced gor-mahń-ē) in honor of George W. Gorman, who isolated LS-13 and was a pioneer in the isolation of legionellae from environmental and clinical sources. The type strain of *L. gormanii* is LS-13 (ATCC 33297). Ordinarily, species are not named on the basis of a single strain, mainly because nothing definitive can be said about the biochemical reactions of a single strain. In this case, because none of the *Legionella* species can be identified solely by biochemical reactions, because of the potential clinical significance of this organism, and because there is no doubt on the basis of DNA relatedness data that this organism represents a new species, we believe that an exception to this rule is justified.

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