Ten New Species of *Legionella*

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Ten new *Legionella* species were characterized on the basis of biochemical reactions, antigens, cellular fatty acids, isoprenoid quinones, and deoxyribonucleic acid relatedness. Nine of the new species were isolated from the environment, and one, *Legionella hackeliae*, was isolated from a bronchial biopsy specimen obtained from a patient with pneumonia. The species all exhibited the following biochemical reactions typical of the legionellae: growth on buffered cysteine-yeast extract agar, but not on blood agar; growth requirement for cysteine; gram negative; nitrate negative; urease negative; nonfermentative; catalase positive; production of a brown pigment on tyrosine-containing yeast extract agar; liquefaction of gelatin; and motility. *Legionella spiritensis* was weakly positive for hydrolysis of hippurate; the other species were hippurate negative. *Legionella cherrii*, *Legionella steigerwaltii*, and *Legionella parisiensis* exhibited bluish white autofluorescence. *Legionella rubrilucens* and *Legionella erythra* exhibited red autofluorescence. The other species, *L. spiritensis*, *L. hackeliae*, *Legionella maceachernii*, *Legionella jamestowniensis*, and *Legionella santacrucis* did not autofluoresce bluish white or red. All species had cellular fatty acid contents qualitatively similar to those of previously described legionellae and had major amounts of ubiquinones with more than 10 isoprene units in the side chains. Each new species was serologically distinct from previously described *Legionella* species. As determined by the hydroxyapatite method at 60°C, two strains of *L. maceachernii* were 100% related, and four strains of *L. cherrii* were 94 to 99% related. The other new species were represented by single strains. The levels of relatedness of the new species to each other and to previously described legionellae ranged from 1 to 67%. *L. maceachernii*, *L. jamestowniensis*, and *L. hackeliae* were less than 25% related to other species. *L. rubrilucens* and *L. erythra*, and two red-autofluorescing species, were about 60% interrelated. *L. spiritensis* (a non-autofluorescing species) was 34% related to *L. rubrilucens*. *L. santacrucis* was 64% related to *Legionella sainthenlei*. The three bluish white-autofluorescing species, *L. parisiensis*, *L. cherrii*, and *L. steigerwaltii*, were most closely related to other bluish white-autofluorescing species, especially *Legionella bozemanii*, *Legionella dumoffii*, *Legionella gormanii*, and "*Legionella anisa*" (35 to 67%).

A total of 221 cases of pneumonia resulting in 34 deaths occurred during an epidemic at the American Legion Convention in Philadelphia, Pa., in 1976 (22, 23). After a massive investigation of this epidemic, the etiological agent of the so-called Legionnaires disease was finally isolated by McDade et al. (36). This organism was shown to be a bacterium that was phenotypically unique, whose deoxyribonucleic acid (DNA) was unrelated to the DNAs of all other species tested (10-12, 45). Therefore, it was classified in a new genus and species, *Legionella pneumophila*, in the new family Legionellaceae (10). *L. pneumophila* was subsequently shown to be a major cause of both pneumonia and an acute, self-limiting febrile disease called Pontiac fever (31). The public health significance of Legionnaires disease stimulated many intense investigations. It was soon learned that *L. pneumophila* had first been isolated in 1947 (35; E. B. Jackson, T. T. Crocker, J. E. Smadel, Bacteriol. Proc., p. 119, 1952). We now recognize at least 10 serogroups in *L. pneumophila* (2, 4, 19, 38, 39; W. F. Bibb and R. M. McKinney, unpublished data). *L. pneumophila* strains were isolated from patients with pneumonia and from the environment.

Strains that resembled *L. pneumophila* but differed in one or more phenotypic characteristics were called *Legionella*-like organisms. Representative strains of *Legionella*-like

organisms were studied biochemically, serologically, chemically for total cellular fatty acids, and by DNA relatedness. New species were proposed, as indicated by these data, with a present total of 12 named species. *Legionella bozemanii* (6, 9, 27) was first isolated in 1959 and *Legionella micdadei* (27, 28, 48, 50) was first isolated in 1943; both of these species have been isolated from patients with pneumonia. *L. micdadei* has also been isolated from human blood and from water and is commonly referred to as the Pittsburgh pneumonia agent (48). *Legionella dumoffii* was isolated first from water and later from human lung tissue (9, 33). *Legionella gormanii* (18, 41) was isolated from water, but serological evidence implicated it as another agent of Legionnaires disease. *Legionella longbeachae* (37) has only been isolated from humans, with strains of this species falling into two serogroups (3, 37). *Legionella jordanis* has been implicated in human pneumonia by indirect fluorescent antibody reactions with human sera (15) and was recently isolated from human lung tissue (51); it has also been isolated from water and sewage. *Legionella oakridgensis* (47) and *Legionella sainthenlei* (14) were isolated from water and have not been isolated from or implicated in cases of human pneumonia. *Legionella wadsworthii* (19) was isolated from a patient with pneumonia. *Legionella feeleyi* was isolated from an industrial water source in a factory where an outbreak of Pontiac fever occurred (29); it was not isolated from patients in this outbreak, but serological evidence indicated that *L. feeleyi* was the causative agent. *L. feeleyi* was also recently isolated

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TABLE 1. *Legionella* strains

Strain	Source	Location	Method ^a	Isolated by:	G+C content (mol %)
<i>L. pneumophila</i> Philadelphia 1 ^T (= ATCC 33152 ^T)	Lung tissue, human pneumonia	Philadelphia, Pa.	Guinea pig→egg→MH1H	J. E. McDade and R. E. Weaver (28 February 1977)	39(10), 39(4) ^b
<i>L. bozemanii</i> WIGA ^T (= ATCC 33217 ^T)	Lung tissue, human	Key West, Fla.	Guinea pig→egg→CYE	F. M. Bozeman (G. A. Hébert) 1957 (25 May 1979)	43(35), 41(13)
<i>L. micdadei</i> TATLOCK ^T (= ATCC 33218 ^T)	Blood, human	Fort Bragg, N.C.	Egg→CYE	H. Tatlock (G. A. Hébert) 1943 (25 May 1979)	44(4), 41
<i>L. dumoffii</i> NY-23 ^T (= ATCC 33279 ^T)	Water, cooling tower	New York, N.Y.	Guinea pig→egg→CYE	G. W. Gorman (6 October 1978)	41(13), 42
<i>L. gormanii</i> LS-13 ^T (= ATCC 33297 ^T)	Soil, creek bank	Atlanta, Ga.	Guinea pig→egg→CYE	G. W. Gorman (1 December 1978)	41(13), 41
<i>L. longbeachae</i> Long Beach 4 ^T (= ATCC 33462 ^T)	Transtracheal aspirate, human pneumonia (fatal)	Long Beach, Calif.	Direct plating on CYE	R. Porschen (10 April 1980)	40
<i>L. jordanis</i> BL-540 ^T (= ATCC 33623 ^T)	Water, river	Bloomington, Ind.	Guinea pig→egg→CYE	G. W. Gorman (1 September 1978)	45
<i>L. oakridgensis</i> Oak Ridge 10 ^T (= ATCC 33761 ^T)	Water, cooling tower	Pennsylvania	Guinea pig→CYE	R. L. Tyndall, C. B. Duncan, and E. L. Domingue (April 1981)	45(47)
<i>L. wadsworthii</i> 81-716 ^T (= ATCC 33877 ^T)	Sputum, human pneumonia	Los Angeles, Calif.	Direct plating on selective BCYE	P. H. Edelstein (23 November 1981)	42(19)
<i>L. feeleyi</i> WO-44C-C3 ^T (= ATCC 35072 ^T)	Water	Windsor, Canada	AT, direct plating on BCYE + AB	G. W. Gorman (August 1981)	46(29)
<i>L. sainthelensi</i> Mount Saint Helens 4 ^T (= ATCC 35248 ^T)	Water, spring	Mount Saint Helens, Wash.	Direct plating on BCYE	J. Campbell and S. Eng (April 1981)	41(14)
" <i>L. anisa</i> " WA-316-C3 ^T (= ATCC 35292 ^T)	Hot water, sink	Los Angeles, Calif.	AT, direct plating on CYE	G. W. Gorman (22 April 1981)	42 ^c
<i>L. maceachernii</i> PX-1-G2-E2 ^T (= ATCC 35300 ^T)	Water, home evaporator-cooler	Phoenix, Ariz.	Guinea pig→egg→CYE	G. W. Gorman (9 July 1979)	43
<i>L. maceachernii</i> SC-73-C2	Potable water cistern	St. Thomas, V.I.	AT, direct plating on CYE	G. W. Gorman (24 April 1982)	41
<i>L. jamestowniensis</i> JA-26-G1-E2 ^T (= ATCC 35298 ^T)	Wet soil	Jamestown, N.Y.	Guinea pig→egg→CYE	G. W. Gorman (12 December 1979)	42
<i>L. rubrilucens</i> WA-270A-C2 ^T (= ATCC 35304 ^T)	Tap water	Los Angeles, Calif.	AT, direct plating on CYE	G. W. Gorman (10 June 1980)	52
<i>L. erythra</i> SE-32A-C8 ^T (= ATCC 35303 ^T)	Water, cooling tower	Seattle, Wash.	AT, direct plating on BCYE	G. W. Gorman (19 January 1981)	51
<i>L. hackeliae</i> Lansing 2 ^T (= ATCC 35250 ^T)	Bronchial biopsy, human pneumonia	Ann Arbor, Mich.	Direct plating on BCYE	M. Hackel (21 July 1981)	40

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TABLE 1—Continued

Strain	Source	Location	Method ^a	Isolated by:	G+C content (mol %)
<i>L. spiritensis</i> Mount Saint Helens 9 ^T (= ATCC 35249 ^T)	Water, lake	Spirit Lake, Wash.	Direct plating on BCYE	J. Campbell (23 August 1981)	46
<i>L. parisiensis</i> PF-209C-C2 ^T (= ATCC 35299 ^T)	Water, cooling tower	Paris, France	AT, direct plating on CYE + AB	G. W. Gorman (9 September 1981)	42
<i>L. cherrii</i> ORW ^T (= ATCC 35252 ^T)	Water, thermally altered	Minnesota	Guinea pig→CYE	R. L. Tyndall and C. B. Duncan (8 January 1982)	40
<i>L. cherrii</i> ORB	Water, thermally altered	Minnesota	Guinea pig→CYE	R. L. Tyndall and C. B. Duncan (8 January 1982)	40
<i>L. cherrii</i> ORZ	Water, thermally altered	Minnesota	Guinea pig→CYE	R. L. Tyndall and C. B. Duncan (8 January 1982)	39
<i>L. cherrii</i> SC-65-C3	Potable water cistern	St. Croix, V.I.	AT, direct plating on CYE	G. W. Gorman (21 March 1982)	
<i>L. steigerwaltii</i> SC-18-C9 ^T (= ATCC 35302 ^T)	Tap water	St. Croix, V.I.	AT, direct plating on CYE + AB	G. W. Gorman (10 March 1982)	40
<i>L. santicrucis</i> SC-63-C7 ^T (= ATCC 35301 ^T)	Tap water	St. Croix, V.I.	AT, direct plating on CYE + AB	G. W. Gorman (21 March 1982)	38

^a MHIH, Mueller-Hinton agar supplemented with IsoVitalX and hemoglobin; selective BCYE, BCYE agar supplemented with 0.1% alpha-ketoglutarate, 4 µg of cefamandole per ml, 80 µg of polymyxin B per ml, and 80 µg of anisomycin per ml; AB, supplemented with 0.5 µg of vancomycin per ml, 4 µg of cephalothin per ml, and 16 µg of colistin per ml; AT, acid treatment.

^b The numbers in parentheses are reference numbers.

^c Data from Gorman et al., in press.

from human sputum (51). “*Legionella anisa*” was isolated from potable water in two hospitals and from water in a cooling tower (G. W. Gorman, J. C. Feeley, A. G. Steigerwalt, P. H. Edelstein, C. W. Moss, and D. J. Brenner, Appl. Environ. Microbiol., in press). Serological studies to assess its possible role in human infection have not been done.

In this report, we summarize the data used to characterize additional legionellae, and we propose names for 10 new species; 9 of these were isolated from the environment, and 1 was isolated from a patient with pneumonia.

MATERIALS AND METHODS

Bacterial strains. The strains used in this study are listed in Table 1. They included the type strains of the 12 previously described *Legionella* species and the type strains of the 10 new species described in this paper. American Type Culture Collection numbers are given for all type strains. Strains of the new species were isolated by one of the following methods (Table 1): (i) direct plating of concentrated water samples or bronchial biopsy material onto charcoal-yeast extract (CYE) agar (21), onto CYE or buffered charcoal-yeast extract (BCYE) agar containing antibiotics (Table 1), or onto BCYE agar (48); (ii) passage of concentrated water samples through guinea pigs, followed by inoculation of infected guinea pig tissues into embryonated hen eggs and subsequent plating of infected yolk sac material onto CYE agar (36, 40, 46); (iii) passage of concentrated water samples through guinea pigs and direct inoculation of infected guinea pig tissue onto CYE agar (40, 46); or (iv) filter concentration of environmental water samples, followed by acid buffer treatment (5, 25). Strains were cultured and maintained on CYE or BCYE agar at 36 ± 1°C in air containing 2.5% CO₂.

Cultural and biochemical tests. All strains of the new *Legionella* species and the type strains of the previously described *Legionella* species were stained by the Hucker modification of the Gram stain. Flagellum staining was done by the Clark modification of the Leifson method (17) or as described by Kodaka et al. (32), and 24- to 48-hour cultures grown on CYE agar at 35°C were examined for motility by dark-field microscopy. Cultures were plated onto BCYE agar with and without L-cysteine and onto Trypticase soy agar supplemented with 5% sheep blood. Autofluorescence was measured by excitation at a wavelength region near 366 nm (54) by using a Woods lamp on cultures grown at 36 ± 1°C on BCYE agar. Formation of a brown, water-soluble pigment was observed on cultures grown at 35°C on tyrosine-containing yeast extract agar (1). Oxidase, catalase, and D-glucose fermentation tests were done as described by Weaver and Feeley (54). Nitrate reduction, gelatin and liquefaction, and urea hydrolysis were tested as described by Orrison et al. (47). Hydrolysis of sodium hippurate was tested by the method of Hébert (26). Beta-lactamase production was assayed by the chromogenic cephalosporin test described by Thornsberry and Kirven (53).

Direct immunofluorescence assays. Formalin-fixed cells of each type strain of the 10 new *Legionella* species were stained with direct immunofluorescence assay conjugates prepared for the detection of all but one of the previously described species and serogroups (16). Included were conjugates for *L. pneumophila* serogroups 1 through 9, *L. longbeachae* serogroups 1 and 2, *L. bozemanii*, *L. micdadei*, *L. dumoffii*, *L. gormanii*, *L. jordanis*, *L. oakridgensis*, *L. wadsworthii*, *L. sainthelensi*, and *L. feeleyi*. A conjugate for “*L. anisa*” was not tested. The strains were also tested with

conjugates prepared for the following three new species described in this study: *Legionella spiritensis* (strain Mount Saint Helens 9^T [T = type strain]), *Legionella hackeliae* (strain Lansing 2^T), and *Legionella rubrilucens* (strain WA-270A-C2^T).

Cellular fatty acids and isoprenoid quinones. The cellular fatty acid compositions and isoprenoid quinone contents were determined as described previously (43–45).

DNA studies. The guanine-plus-cytosine (G+C) contents of *Legionella* DNAs were determined spectrophotometrically by the thermal denaturation method (34). DNAs from *Proteus mirabilis* 1 (40 mol% G+C) and *Escherichia coli* K-12 (51 mol% G+C) were used as controls. For the preparation of DNA, strains were grown on BCYE agar in 15 to 30 petri plates (100 by 15 mm). The plates were incubated at 35°C in an atmosphere containing 2.5% CO₂ until growth was confluent (3 to 5 days). The preparation of unlabeled and ³²PO₄-in-vitro-labeled DNA has been described previously, as has the determination of DNA relatedness by the hydroxyapatite method at 60 and 75°C (12).

RESULTS

Legionella and *Legionella*-like organisms are received for identification at the Centers for Disease Control from state and federal laboratories and from laboratories outside the United States or are obtained in the course of disease outbreaks and environmental studies. These strains are first studied biochemically and serologically. If these methods fail to identify strains as members of recognized *Legionella* species, the strains are examined for their cellular fatty acid and isoprenoid quinone contents and are characterized further by serological testing and DNA hybridization. The results of these studies allow assignment of such strains to new species or new serogroups in existing species or iden-

tification as atypical members of existing species. This experimental protocol was used to characterize all of the presently described species and serogroups in the genus *Legionella*. The most recent of these are serogroups 7 and 8 in *L. pneumophila* (2, 4), a second serogroup in *L. bozemanii* (49), and the new species *L. feeleii* (29), *L. sainthelensi* (14), and "*L. anisa*" (Gorman et al., in press).

Similar studies are described here for 14 strains that represent 10 new species in the genus *Legionella*. These species were first isolated between July 1979 and March 1982. *L. hackeliae* was isolated from a bronchial biopsy specimen obtained from a woman with pneumonia. All other species were isolated from various water sources (Table 1).

Biochemical studies. The biochemical characteristics of the new *Legionella* species are shown in Table 2. These characteristics are extremely similar to those of the previously described legionellae. *L. spiritensis* and perhaps *L. feeleii* are the only new species that hydrolyze hippurate, as most strains of *L. pneumophila* do. The oxidase reaction varied among the new species, as it did among previously described species. Five of the new species autofluoresced when cultures were examined under a Woods light. Three of these, *Legionella parisiensis*, *Legionella cherrii*, and *Legionella steigerwaltii*, exhibited bluish white autofluorescence similar to that observed with *L. bozemanii*, *L. dumoffii*, *L. gormanii*, and "*L. anisa*." *L. rubrilucens* and *Legionella erythra* exhibited red autofluorescence.

Cellular fatty acid and isoprenoid quinone analyses. Like other legionellae, each of the 10 new species contained major amounts of ubiquinones with more than 10 isoprene units in the side chain (14, 30, 43). The cellular fatty acids of the 10 new species were qualitatively similar to those of the previously described legionellae (14, 15, 19, 27, 42, 43, 47;

TABLE 2. Phenotypic characteristics of legionellae^a

Species	Growth on blood agar	Growth on BCYE agar	Growth requirement for cysteine	Gram stain	NO ₂	Urease	Acid from D-glucose	Catalase	Browning of YE containing tyrosine ^b	Gelatin liquefaction	Hippurate hydrolysis	Motility	Oxidase	Beta-lactamase	Auto-fluorescence
<i>L. pneumophila</i>	— ^c	+	+	—	—	—	—	+	+	+	+	+	+ or +/-	+	—
<i>L. feeleii</i>	—	+	+	—	—	—	—	+	+(w)	—	+/-	+	—	—	—
<i>L. spiritensis</i>	—	+	+	—	—	—	—	+	+	+	+(w)	+	+	+	—
<i>L. longbeachae</i>	—	+	+	—	—	—	—	+	+	+	—	+	+	+/-	—
<i>L. jordanii</i>	—	+	+	—	—	—	—	+	+	+	—	+	+	+	—
<i>L. oakridgensis</i>	—	+	+ ^d	—	—	—	—	+	+	+	—	—	—	+(w)	—
<i>L. wadsworthii</i>	—	+	+	—	—	—	—	+	—	+	—	+	—	+	—
<i>L. sainthelensi</i>	—	+	+	—	—	—	—	+	+	+	—	+	+	+	—
<i>L. hackeliae</i>	—	+	+	—	—	—	—	+(w)	+	+	—	+	+	+	—
<i>L. maceachernii</i>	—	+	+	—	—	—	—	—	+	+	—	+	+	—	—
<i>L. jamestowniensis</i>	—	+	+	—	—	—	—	+	+	+	—	+	—	+	—
<i>L. santicrucis</i>	—	+	+	—	—	—	—	+	+	+	—	+	+	+	—
<i>L. micdadei</i>	—	+	+	—	—	—	—	+	—	—	—	+	+	—	—
<i>L. bozemanii</i>	—	+	+	—	—	—	—	+	+	+	—	+	+/-	+/-	+(BW)
<i>L. dumoffii</i>	—	+	+	—	—	—	—	+	+ ^e	+	—	+	—	+	+(BW)
<i>L. gormanii</i>	—	+	+	—	—	—	—	+	+	+	—	+	—	+	+(BW)
" <i>L. anisa</i> "	—	+	+	—	—	—	—	+	+	+	—	+	+	+	+(BW) ^f
<i>L. cherrii</i>	—	+	+	—	—	—	—	+	+	+	—	+	—	+	+(BW)
<i>L. steigerwaltii</i>	—	+	+	—	—	—	—	+	+	+	—	+	—	+	+(BW)
<i>L. parisiensis</i>	—	+	+	—	—	—	—	+	+	+	—	+	+	+	+(BW)
<i>L. rubrilucens</i>	—	+	+	—	—	—	—	+	+	+	—	+	—	+	+(R)
<i>L. erythra</i>	—	+	+	—	—	—	—	+	+	+	—	+	+	+	+(R)

^a Data for previously described species were taken from the following references: 9, 14, 15, 19, 26–29, 33, 37, 41, 47, 52–54; Gorman et al., in press.

^b YE, Charcoal-treated yeast extract agar.

^c +, Positive; —, negative; +(w), weak reaction; +/-, Weakly or not always positive; (BW), bluish white autofluorescence; (R), red autofluorescence.

^d Strains of this species adapt to grow in the absence of cysteine, but require cysteine when first isolated.

^e A strain of *L. dumoffii* did not produce a diffusible brown pigment (P. H. Edelstein, personal communication).

^f One of the seven known "*L. anisa*" strains does not autofluoresce (Gorman et al., in press).

Gorman et al., in press). With the exception of *L. erythra*, all of the new species contained major amounts of branched-chain fatty acids. The cellular fatty acids and ubiquinones of these species will be described separately in detail (C. W. Moss, manuscript in preparation).

Characterization by direct immunofluorescence staining. Conjugates prepared for *L. spiritensis*, *L. hackeliae*, and *L. rubrilucens* stained their respective type strains (strains Mount Saint Helens 9, Lansing 2, and WA-270A-C2) with 4+ fluorescence intensity, and cross-reactions with other *Legionella* species were not observed. Some cross-reactions were observed between conjugates for previously characterized legionellae and antigens prepared from type strains of the 10 new species. *L. parisiensis* stained weakly with conjugates for *L. bozemanii* (fluorescence intensity, 1+), *L. micdadei* (1+), and *L. jordanis* (2+); *Legionella santicrucis* stained 3+ with the *L. longbeachae* serogroup 1 conjugate. The only additional cross-reactions observed when undiluted conjugates (10 mg of immunoglobulin G per ml of conjugate) were used were between *L. santicrucis* antigen and *L. sainthelensi* conjugate (2+) and between *Legionella maceachernii* antigen and *L. micdadei* conjugate (3+). The weak cross-reactions of *L. parisiensis* antigen with *L. bozemanii* and *L. jordanis* became 4+ when undiluted conjugates were used. Antigenic relationships were noted previously for *L. bozemanii* and *L. jordanis* (15) and for *L. longbeachae* and *L. sainthelensi* (14). Comprehensive antigenic analyses of the new species are in progress (H. W. W.).

DNA studies. DNAs from all of the new type strains representing *Legionella* species were labeled in vitro with ^{32}P . DNA hybridization reactions were done to establish the uniqueness of each species and to determine its relatedness to all other *Legionella* species (Table 3). Type strains of all species, as well as additional strains of the new species, where available, were used as sources of the unlabeled DNAs in these experiments.

Legionella jamestowniensis was 1 to 17% related to other *Legionella* species. *L. hackeliae* was 3 to 22% related to other species, with 14 to 17% relatedness (in reciprocal reactions) to *L. jamestowniensis* and 22% relatedness to *L. cherrii*. *L. maceachernii* consisted of two strains that were essentially identical; its closest relative (23%) was *L. micdadei*. *L. spiritensis* was most related to *L. rubrilucens* (34%), a red-autofluorescing species, and was 19 to 21% related to a number of species, including *L. erythra*, the other red-autofluorescing species.

The two red-autofluorescing species, *L. rubrilucens* and *L. erythra*, were 59 to 62% interrelated, with 9.0 to 9.5% divergence in related sequences, and 27 to 34% related in 75°C reactions; their closest relative was *L. spiritensis* (34 and 20% related, respectively).

The three bluish white-autofluorescing species, *L. parisiensis*, *L. cherrii*, and *L. steigerwaltii*, were substantially related to one another and to the other bluish white-autofluorescing species. *L. parisiensis* was 57 to 67% related to four strains of *L. bozemanii*, with 7.5 to 9.0% divergence in related sequences, and 26 to 39% related in 75°C reactions. *L. parisiensis* was 50 to 59% related to three "*L. anisa*" strains and 22 to 40% related to the other bluish white-autofluorescing species. The level of relatedness of *L. parisiensis* to species that do not exhibit bluish white autofluorescence was highest with *L. wadsworthii* (37%). The four strains of *L. cherrii* were 94% or more related at optimal (60°C) and stringent (75°C) reassociation criteria and exhibited 0.0% to 0.5% divergence in related sequences. *L. cherrii* was highly related to *L. steigerwaltii* (67%), *L. dumoffii*

(57%), "*L. anisa*" (56%), *L. bozemanii* (51%), and *L. gormanii* (47%). The level of relatedness of *L. cherrii* to another bluish white-autofluorescing species, *L. parisiensis*, was 24%. *L. cherrii* was 6 to 35% related to other legionellae. *L. steigerwaltii* was 32 to 54% related to the other bluish white-autofluorescing species and 31 to 34% related to the non-autofluorescing species *L. santicrucis*, *L. wadsworthii*, and *L. sainthelensi*.

L. santicrucis was closely related to *L. sainthelensi* (64% at 60°C, 23% at 75°C) and was 38% related to *L. longbeachae*.

It is clear that each of the 10 new *Legionella* species, as well as the 12 previously described species, can be separated from all other legionellae on the basis of DNA relatedness. The strains of any one species showed 70% or more relatedness in 60°C reactions, less than 4% divergence in related sequences, and more than 60% relatedness in 75°C reactions.

The 12 previously described *Legionella* species had G+C contents of 39 to 46 mol% (Table 1), and 8 of the 10 new species had G+C contents of 38 to 46 mol% (Table 1). The two red-autofluorescing species, *L. parisiensis* and *L. erythra*, had G+C contents of 52 and 51 mol%, respectively (Table 1).

DISCUSSION

Brown and colleagues proposed the formation of the following three genera from the first five species of *Legionella* (13, 24): *Legionella* (with only one species, *L. pneumophila*), *Tatlockia* (with *Tatlockia micdadei*), and *Fluoribacter* (with *Fluoribacter bozemanii*, *Fluoribacter dumoffii*, and *Fluoribacter gormanii*). We have opposed this proposal on several grounds. First, there is not a genetic definition for a genus. If there were one, it is doubtful that the relatedness values among the three species that were placed in *Fluoribacter* would fit the genetic definition of a genus. The level of relatedness between *Fluoribacter* species is as low as 12%, with as much as 25% relatedness between these species and *L. pneumophila* in our studies (9, 10, 21, 41). In the studies of Brown et al., *Fluoribacter* species showed as little as 4% interrelatedness, compared with values of up to 14% with *L. pneumophila* and up to 10% with *L. micdadei* (13, 24).

If we consider the creation of additional genera for not only the first 5 species but all 22 *Legionella* species, the problems are compounded (Table 4). At a 40% relatedness level, there are 14 genera; one contains the 7 bluish white-autofluorescing species, one contains the 2 red-autofluorescing species, 1 contains *L. sainthelensi* and *L. santicrucis*, and 11 genera each contain a single species. This 40% criterion ignores the fact that several of the species in the bluish white-autofluorescing group are not 40% related to all of the other species in the group and the fact that *L. wadsworthii* is as much as 37% related to members of this group.

At a 30% relatedness level there would be 11 genera. *L. spiritensis* (which does not autofluoresce) is added to the red-autofluorescing group. *L. wadsworthii* (which does not autofluoresce) is added to the bluish white-autofluorescing group. *L. santicrucis* and *L. sainthelensi* (which do not autofluoresce) are also more than 30% related to the bluish white-autofluorescing group, but they also form their own group, along with *L. longbeachae* and two of the bluish white-autofluorescing species, *L. steigerwaltii* and *L. cherrii*. Eight additional genera would contain single species.

At a 25% relatedness level, as advocated by Brown and colleagues (13, 24), there would be nine genera. The red-au-

tofluorescing group remains the same as at the 30% level, the bluish white-autofluorescing group and the *L. sainthelensi* group merge into a single genus, a new group is formed (at a relatedness level of 23%) from *L. micdadei* and *L. maceachernii*, and six genera contain single species.

Even if one could define and separate these genetic genera by phenotypic tests, none of these alternatives is appealing. The fact is that legionellae can be identified to species only by serogrouping or DNA hybridization. With a combination of biochemical tests and analyses of cellular fatty acids and ubiquinones, one can define legionellae at the family level and form species groups, but cannot identify individual species.

Very few strains of most *Legionella* species have been examined phenotypically; in many species only one or a few strains have been isolated. Even with this limited sample, there is phenotypic variation. Not all strains of *L. pneumophila* hydrolyze hippurate (26), one *L. dumoffii* strain does not produce brown pigment (P. H. Edelstein, personal communication), one strain of "*L. anisa*" does not exhibit bluish white autofluorescence (Gorman et al, in press), and the *L. feeleyi* strain has been reported to hydrolyze hippurate (29) but did not do so when retested as part of this study (Table 2). Furthermore, grouping of species on the basis of DNA relatedness creates problems due to mixed phenotypes. *L. wadsworthii*, *L. santicrucis*, *L. sainthelensi*, and *L. longbeachae* do not autofluoresce, but depending on the DNA criteria used to define a genus, they could be grouped with the bluish white-autofluorescing species. Similarly, *L. spiritensis* could be grouped with the red-autofluorescing species.

There is a good definition for strains of a genetic species (70% or more relatedness in reactions done at optimal criteria for DNA reassociation, 6% or less divergence in related sequences, and at least 55% relatedness in reactions done at a supraoptimal [stringent] criterion for DNA reassociation [7]). It is our opinion that although a species reflects evolutionary divergence, a genus is a man-made division that should consist of a group of phenotypically similar species that must be differentiated from one another at the microbiology bench. If genetic and phenotypic similarities are not in agreement, then a genus based on phenotype is preferable and more practical to taxonomists and to all microbiologists than a genus based on genetic relatedness. *Legionella* is certainly a good phenotypic genus. All species are isolated by the same methods and cultivated on the same media. They have similar biochemical profiles and are easily differentiated from other gram-negative bacteria by their unique fatty acid composition and isoprenoid quinone content. When motile, they have a common flagellar antigen. Many species exhibit cross-reactions between their somatic antigens. All species that have been isolated from humans cause the same disease, and the same treatment regimen is used against all species known to cause human diseases. For all of the above reasons it would be of little, if any, benefit to create additional genera in the family *Legionellaceae* at this time.

Taxonomic proposals. Nomenclatural proposals are given below for 10 new *Legionella* species; 3 of the 10 new species are named after people who are coauthors of this paper. In all of these cases, the names were arrived at by D.J.B. and G.W.G. without the knowledge of the people for whom the species are named.

The 10 new species conform to the definition of the family *Legionellaceae* and to the definition of the genus *Legionella* (8), except that the range of G+C contents within the family

and the genus is now 38 to 52 mol%. All of the species are gram-negative, catalase-positive rods that grow on BCYE agar but not on blood agar and require cysteine for growth. They are motile by means of one to a few polar, subpolar, or lateral flagella. They do not produce acid from D-glucose, reduce nitrates, or contain a urease. They liquefy gelatin, and they form a soluble brown pigment on yeast extract agar that contains tyrosine. Unless noted below, the new species do not hydrolyze hippurate, and they do produce beta-lactamase. The sources of the strains of each species are given in Table 1, and their DNA relatedness relationships are shown in Table 3. Other characteristics of the 10 new species are given below and in the tables and text.

***Legionella* species 13: *Legionella maceachernii*.** *Legionella maceachernii* sp. nov. (mac. kath'er. ni. i. N. L. gen. n. *maceachernii* in honor of Harold V. McEachern who has provided dedicated laboratory support from the earliest studies on legionellae to the present and as a tribute to the dozens of dedicated laboratory personnel who have contributed so much to our knowledge of legionellae) is an oxidase-positive organism that does not autofluoresce or produce beta-lactamase. Its other biochemical reactions are typical for legionellae, as described above. The type strain of *L. maceachernii* is strain PX-1-G2-E2 (= ATCC 35300), which has a G+C content of 43 mol%.

***Legionella* species 14: *Legionella jamestowniensis*.** *Legionella jamestowniensis* sp. nov. (james. town' i. en. sis. N. L. fem. adj. *jamestowniensis* coming from Jamestown, N.Y.) is an oxidase-negative organism that does not autofluoresce. Its other biochemical reactions are typical for legionellae, as described above. The type strain is strain JA-26-G1-E2 (= ATCC 35298), which has a G+C content of 42 mol%.

***Legionella* species 15: *Legionella rubrilucens*.** *Legionella rubrilucens* sp. nov. (ru. bri. lu' cens. N. L. part adj. *rubrilucens* red-shining, referring to red autofluorescence) is an oxidase-negative organism that autofluoresces red. Its other biochemical reactions are typical for legionellae, as described above. The type strain is strain WA-270A-C2 (= ATCC 35304), which has a G+C content of 52 mol%.

***Legionella* species 16: *Legionella erythra*.** *Legionella erythra* sp. nov. (e. ry' thra. Gr. fem. adj. *erythra* red, referring to red autofluorescence) is an oxidase-positive organism that autofluoresces red. Its other biochemical reactions are typical for legionellae, as described above. The type strain is strain SE-32A-C8 (= ATCC 35303), which has a G+C content of 51 mol%.

***Legionella* species 17: *Legionella hackeliae*.** *Legionella hackeliae* sp. nov. (hack' el. i. ae. N. L. gen. n. *hackeliae* in honor of Meredith Hackel, who isolated it, and in tribute to the many state public health and clinical laboratory workers who have isolated new strains of legionellae) is an oxidase-positive organism that does not autofluoresce. Its other biochemical reactions are typical for legionellae, as described above. The type strain is strain Lansing 2 (= ATCC 35250), which has a G+C content of 40 mol%.

***Legionella* species 18: *Legionella spiritensis*.** *Legionella spiritensis* sp. nov. (spir' it. en. sis. N. L. fem. adj. *spiritensis* coming from Spirit Lake) is an oxidase-positive organism that gives a weakly positive reaction in the test for hydrolysis of hippurate and does not autofluoresce. Its other biochemical reactions are typical for legionellae, as described above. The type strain is strain Mount Saint Helens 9 (= ATCC 35249), which has a G+C content of 46 mol%.

***Legionella* species 19: *Legionella parisiensis*.** *Legionella parisiensis* sp. nov. (pa. ri. si. en' sis. N. L. fem. adj. *parisiensis* coming from Paris, France) is an oxidase-positive

TABLE 3. DNA relatedness of 10 new *Legionella* species^a

Source of unlabeled DNA	<i>L. maceachernii</i> PX-1-G2-E2 ^T		<i>L. james-townensis</i> JA-26-G1-E2 ^T		<i>L. rubrilucens</i> WA-270A-C2 ^T		<i>L. erythra</i> SE-32A-C8 ^T		<i>L. hackeliae</i> Lansing 2 ^T		<i>L. spiritalensis</i> Saint Helens 9 ^T		<i>L. parisiensis</i> PF-209C-C2 ^T		<i>L. cherrii</i> ORW ^T		<i>L. steigerwaltii</i> SC-18-C9 ^T		<i>L. santacrucis</i> SC-63-C7 ^T		
	RBR at 60°C ^b	D (%) ^c	RBR at 60°C	RBR at 60°C	RBR at 60°C	D (%)	RBR at 60°C	D (%)	RBR at 60°C	D (%)	RBR at 60°C	D (%)	RBR at 60°C	D (%)	RBR at 60°C	D (%)	RBR at 60°C	D (%)	RBR at 60°C	RBR at 75°C	
<i>L. maceachernii</i> PX-1-G2-E2 ^T	100	0.0	100	3	9		13		6		19	10				8			12		
<i>L. maceachernii</i> SC-73-C2	100	0.0	98					1													
<i>L. james-townensis</i> JA-26-G1-E2 ^T	7		3	100	7		15		17		10	6				6			9		
<i>L. rubrilucens</i> WA-270A-C2 ^T	4			8	100	0.0	100	62	5		34	6				20			10		
<i>L. erythra</i> SE-32A-C8 ^T	6			5	59	9.5	27	100	0.0	100		7				7			10		
<i>L. hackeliae</i> Lansing 2 ^T				14					100		10					22					
<i>L. spiritalensis</i> Saint Helens 9 ^T				6					6		100					16			11		
<i>L. parisiensis</i> PF-209C-C2 ^T	10			5			10		6		4	100	0.0	100	24	32	5	29	11		
<i>L. cherrii</i> ORW ^T	8											28			100	0.0	100				
<i>L. cherrii</i> ORB	6														94	0.0	98				
<i>L. cherrii</i> ORZ	5											22			94	0.0	95				
<i>L. cherrii</i> SC-65-C3				4					4		4	32			99	0.5	100	54	8.5	22	
	9			3					7		6	35			67	5.5	28	100	0.0	19	
<i>L. steigerwaltii</i> SC-18-C9 ^T				4					5		4	25			35			31	14.5	100	
<i>L. santacrucis</i> SC-63-C7 ^T																					
<i>L. pneumophila</i> Philadelphia 1 ^T	5			4	9		6		4		7	11			21			11		7	
<i>L. bozemani</i> WIGA ^T	6			3	6		4		5		6	65 ^d	7.5	26	51	10.5	11	46	12.0	4	20
<i>L. micdadei</i> TATLOCK ^T	23			1	7		4		6		16	5			18			10		10	
<i>L. dumoffii</i> NY-23 ^T	8			2	6		6		9		19	35	12.0	7	57	11.0	8	48	13.0	8	20
<i>L. gormanii</i> LS-13 ^T	8			11	8		5		4		19	40	12.0	7	47			7	40		7

<i>L. longbeachae</i>	3	6	2	2	11	24	22	17.0	38	26
Long Beach 4 ^T										
<i>L. jordanis</i> BL-540 ^T	7	8	10	5	23	2	10		7	
<i>L. oakridgensis</i>	5	9	9 ^c	8	5	6	12		9	
Oak Ridge 10 ^T										
<i>L. wadsworthii</i>	7	3	5 ^f	12	37	10.5	34		4	19
81-716 ^T										
<i>L. feeleeii</i>	9	8	8 ^g	15	5	16	10		9	
WO-44C-C3 ^T										
<i>L. sainthelensi</i>	5	5	5 ^h	7	23	15.0	33	15.0	5	64
Mount Saint Helens 4 ^T										
" <i>L. anisa</i> "	10	5	5	11	56 ^d	9.0	53	12.5	6	25
WA-316-C3 ^T										

^a ³²P- α -labeled DNAs were reacted with unlabeled DNAs from the same strain (homologous reaction) and from a series of other legionellae. Each reaction was done at least twice. Reassociation values in homologous reactions ranged from 45 to 80% before normalization. Control reactions, in which labeled DNA was incubated in the absence of unlabeled DNA, showed 0 to 2.5% binding to hydroxyapatite. These control values were subtracted before normalization.

^b The relative binding ratio (RBR) was calculated as follows: RBR = (percentage of DNA bound to hydroxyapatite in heterologous reaction/Percentage of DNA bound to hydroxyapatite in homologous reaction) $\times$ 100.

^c Percent divergence (D) was calculated on the assumption that a 1% decrease in the thermal stability of a heterologous DNA duplex compared with the thermal stability of the homologous DNA duplex was caused by 1% of the bases within the duplex being unpaired; it was calculated to the nearest 0.5%.

^d The level of relatedness of strain PF-209C-C2^T DNA to the DNAs of three other *L. bozemanii* strains were 57, 63, and 67% at 60°C, with divergence values of 8.0 and 9.0% (two strains) and levels of relatedness in 75°C reactions of 35 and 39% (two strains); the levels of relatedness of strain PF-209C-C2^T DNA to the DNAs of two other "*L. anisa*" strains was 50 and 59% in 60°C reactions.

^e The labeled DNA was from strain Oak Ridge 10^T.

^f The labeled DNA was from strain 81-716^T.

^g The labeled DNA was from strain WO-44C-C3^T.

^h The labeled DNA was from strain Mount Saint Helens 4^T.

TABLE 4. Possible division of legionellae into multiple genera on the basis of DNA relatedness levels

Level of relatedness (%)	Genus designation	Species included
40	I	<i>L. rubrilucens</i> , <i>L. erythra</i>
	II	<i>L. parisiensis</i> , <i>L. cherrii</i> , <i>L. steigerwaltii</i> , <i>L. bozemanii</i> , <i>L. dumoffii</i> , <i>L. gormanii</i> , " <i>L. anisa</i> "
	III	<i>L. sainthelensi</i> , <i>L. santacrucis</i>
	IV	<i>L. spiritensis</i>
	V	<i>L. wadsworthii</i>
	VI	<i>L. longbeachae</i>
	VII	<i>L. micdadei</i>
	VIII	<i>L. maceachernii</i>
	IX	<i>L. pneumophila</i>
	X	<i>L. jordanis</i>
	XI	<i>L. oakridgensis</i>
	XII	<i>L. feeleeii</i>
	XIII	<i>L. jamestowniensis</i>
	XIV	<i>L. hackeliae</i>
30	I	<i>L. rubrilucens</i> , <i>L. erythra</i> , <i>L. spiritensis</i>
	II	<i>L. parisiensis</i> , <i>L. cherrii</i> , <i>L. steigerwaltii</i> , <i>L. bozemanii</i> , <i>L. dumoffii</i> , <i>L. gormanii</i> , " <i>L. anisa</i> ," <i>L. wadsworthii</i> (<i>L. santacrucis</i> , <i>L. sainthelensi</i>) ^a
	III	<i>L. sainthelensi</i> , <i>L. santacrucis</i> , <i>L. longbeachae</i> (<i>L. steigerwaltii</i> , <i>L. cherrii</i>) ^a
	IV	<i>L. micdadei</i>
	V	<i>L. maceachernii</i>
	VI	<i>L. pneumophila</i>
	VII	<i>L. jordanis</i>
	VIII	<i>L. oakridgensis</i>
	IX	<i>L. feeleeii</i>
	X	<i>L. jamestowniensis</i>
	XI	<i>L. hackeliae</i>
25	I	<i>L. rubrilucens</i> , <i>L. erythra</i> , <i>L. spiritensis</i> , <i>L. parisiensis</i> , <i>L. cherrii</i> , <i>L. steigerwaltii</i> , <i>L. bozemanii</i> , <i>L. dumoffii</i> , <i>L. gormanii</i> , " <i>L. anisa</i> ," <i>L. wadsworthii</i> , <i>L. santacrucis</i> , <i>L. sainthelensi</i> , <i>L. longbeachae</i>
	II	
	III	<i>L. micdadei</i> , <i>L. maceachernii</i>
	IV	<i>L. pneumophila</i>
	V	<i>L. jordanis</i>
	VI	<i>L. oakridgensis</i>
	VII	<i>L. feeleeii</i>
	VIII	<i>L. jamestowniensis</i>
	IX	<i>L. hackeliae</i>

^a At this criterion for genus formation the species in parentheses are at or just below the genus level.

organism that autofluoresces bluish white. Its other biochemical reactions are typical for legionellae, as described above. The type strain is strain PF-209C-C2 (= ATCC 35299) which has a G+C content of 42 mol%.

Legionella species 20: *Legionella cherrii*. *Legionella cherrii* sp. nov. (cher' ri. i. N. L. gen. n. *cherrii* in honor of William B. Cherry for his many pioneering contributions to our knowledge of legionellae) is an oxidase-negative organism that autofluoresces bluish white. Its other biochemical reactions are typical for legionellae, as described above. The type strain is strain ORW (= ATCC 35252), which has a G+C content of 40 mol%.

Legionella species 21: *Legionella steigerwaltii*. *Legionella steigerwaltii* sp. nov. (stei. ger. walt' i. i. N. L. gen. n. *steigerwaltii* in honor of Arnold G. Steigerwalt, who did the definitive experiments to classify all 22 known *Legionella*

species) is an oxidase-negative organism that autofluoresces bluish white. Its other biochemical reactions are typical for legionellae, as described above. The type strain is strain SC-18-C9 (= ATCC 35302), which has a G+C content of 40 mol%.

Legionella species 22: *Legionella santicrucis*. *Legionella santicrucis* sp. nov. (san. ti. cru' cis. N. L. gen. n. *santicrucis* of Santa Crux, Latin name of St. Croix, Virgin Islands) is an oxidase-positive organism that does not autofluoresce. Its other biochemical reactions are typical for legionellae, as described above. The type strain is strain SC-63-C7 (= ATCC 35301), which has a G+C content of 38 mol%.

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LITERATURE CITED

- Baine, W. B., J. K. Rasheed, J. C. Feeley, G. W. Gorman, and L. E. Casida, Jr. 1978. Effect of supplemental L-tyrosine on pigment production in cultures of the Legionnaires' disease bacterium. *Curr. Microbiol.* 1:93-94.
- Bibb, W. F., P. M. Arnow, D. L. Dellinger, and S. R. Perryman. 1983. Isolation and characterization of a seventh serogroup of *Legionella pneumophila*. *J. Clin. Microbiol.* 17:346-348.
- Bibb, W. F., R. J. Sorg, B. M. Thomason, M. Hicklin, A. G. Steigerwalt, D. J. Brenner, and M. R. Wulf. 1981. Recognition of a second serogroup of *Legionella longbeachae*. *J. Clin. Microbiol.* 14:674-677.
- Bissett, M. L., J. O. Lee, and D. S. Lindquist. 1983. A new serogroup of *Legionella pneumophila*: serogroup 8. *J. Clin. Microbiol.* 17:887-891.
- Bopp, S., J. W. Sumner, G. K. Morris, and J. G. Wells. 1981. Isolation of *Legionella* spp. from environmental water samples by low-pH treatment and use of selective medium. *J. Clin. Microbiol.* 13:714-719.
- Bozeman, F. M., J. W. Humphries, and J. M. Campbell. 1968. A new group of rickettsia-like agents recovered from guinea pigs. *Acta Virol.* (English ed.) 12:87-93.
- Brenner, D. J. 1981. Introduction to the family *Enterobacteriaceae*, p. 1105-1127. In M. P. Starr, H. Stolp, H. G. Trüper, A. Balows, and H. G. Schlegel (ed.), *The prokaryotes*. Springer-Verlag, New York.
- Brenner, D. J., J. C. Feeley, and R. E. Weaver. 1984. Family VII. *Legionellaceae* Brenner, Steigerwalt, and McDade 1979, 658, p. 279-288. In N. R. Krieg and J. G. Holt (ed.), *Bergey's manual of systematic bacteriology*, vol. 1. The Williams & Wilkins Co., Baltimore.
- Brenner, D. J., A. G. Steigerwalt, G. W. Gorman, R. E. Weaver, J. C. Feeley, L. G. Cordes, H. W. Wilkinson, C. Patton, B. M. Thomason, and K. R. Lewallen Sasseville. 1980. *Legionella bozemanii* sp. nov. and *Legionella dumoffii* sp. nov.: classification of two additional species of *Legionella* associated with human pneumonia. *Curr. Microbiol.* 4:111-116.
- Brenner, D. J., A. G. Steigerwalt, and J. E. McDade. 1979. Classification of the Legionnaires' disease bacterium: *Legionella pneumophila*, genus novum, species nova of the family *Legionellaceae*, familia nova. *Ann. Intern. Med.* 90:656-658.
- Brenner, D. J., A. G. Steigerwalt, S. Pohl, H. Behrens, W. Mannheim, and R. E. Weaver. 1981. Lack of relatedness of *Legionella pneumophila* to *Cytophagaceae*, "Pasteurellaceae", and *Kingella*. *Int. J. Syst. Bacteriol.* 31:89-90.
- Brenner, D. J., A. G. Steigerwalt, R. E. Weaver, J. E. McDade, and J. C. Feeley, and M. Mandel. 1978. Classification of the Legionnaires' disease bacterium: an interim report. *Curr. Microbiol.* 1:71-75.
- Brown, A., G. M. Garrity, and R. M. Vickers. 1981. *Fluoribacter dumoffii* (Brenner et al.) comb. nov. and *Fluoribacter gormanii* (Morris et al.) comb. nov. *Int. J. Syst. Bacteriol.* 31:111-115.
- Campbell, J., W. F. Bibb, M. A. Lambert, S. Eng, A. G. Steigerwalt, J. Allard, C. W. Moss, and D. J. Brenner. 1984. *Legionella sainthelensi*: a new species of *Legionella* isolated from water near Mt. St. Helens. *Appl. Environ. Microbiol.* 47:369-373.
- Cherry, W. B., G. W. Gorman, L. H. Orrison, C. W. Moss, A. G. Steigerwalt, H. W. Wilkinson, S. E. Johnson, R. M. McKinney, and D. J. Brenner. 1982. *Legionella jordanis*: a new species of *Legionella* isolated from water and sewage. *J. Clin. Microbiol.* 15:290-297.
- Cherry, W. B., and R. M. McKinney. 1979. Detection of Legionnaires' disease bacteria in clinical specimens by direct immunofluorescence, p. 91-103. In G. L. Jones and G. A. Hébert (ed.), "Legionnaires", the disease, the bacterium and methodology. Centers for Disease Control, Atlanta.
- Clark, W. A. 1976. A simplified Leifson flagella stain. *J. Clin. Microbiol.* 3:632-634.
- Cordes, L. G., H. W. Wilkinson, G. W. Gorman, B. J. Fikes, and D. W. Fraser. 1979. Atypical *Legionella*-like organisms: fastidious water-associated bacteria pathogenic for man. *Lancet* ii:927-930.
- Edelstein, P. H., D. J. Brenner, C. W. Moss, A. G. Steigerwalt, E. M. Francis, and W. L. George. 1982. *Legionella wadsworthii* species nova: a cause of human pneumonia. *Ann. Intern. Med.* 97:809-813.
- England, A. C., III, R. M. McKinney, P. Skaliy, and G. W. Gorman. 1980. A fifth serogroup of *Legionella pneumophila*. *Ann. Intern. Med.* 93:58-59.
- Feeley, J. C., R. J. Gibson, G. W. Gorman, N. C. Langford, J. K. Rasheed, D. C. Mackel, and W. B. Baine. 1979. Charcoal-yeast extract agar: primary isolation medium for *Legionella pneumophila*. *J. Clin. Microbiol.* 10:436-441.
- Fraser, D. W., and J. E. McDade. 1979. Legionellosis. *Sci. Am.* 241:82-99.
- Fraser, D. W., T. F. Tsai, W. Orenstein, W. E. Parkin, H. J. Beecham, R. G. Sharrer, J. Harris, G. F. Mallison, S. M. Martin, J. E. McDade, C. C. Shepard, P. S. Brachman, and the Field Investigation Team. 1977. Legionnaires' disease: description of an epidemic. *N. Engl. J. Med.* 297:1189-1197.
- Garrity, G. M., A. Brown, and R. M. Vickers. 1980. *Tatlockia* and *Fluoribacter*: two new genera of organisms resembling *Legionella pneumophila*. *Int. J. Syst. Bacteriol.* 30:609-614.
- Gorman, G. W., J. M. Barbaree, and J. C. Feeley. 1983. Procedures for the recovery of *Legionella* from water. Centers for Disease Control, Atlanta.
- Hébert, G. A. 1981. Hippurate hydrolysis by *Legionella pneumophila*. *J. Clin. Microbiol.* 13:240-242.
- Hébert, G. A., C. W. Moss, L. K. McDougal, F. M. Bozeman, R. M. McKinney, and D. J. Brenner. 1980. The rickettsia-like organisms TATLOCK (1943) and HEBA (1959): bacteria phenotypically similar to but genetically distinct from *Legionella pneumophila* and the WIGA bacterium. *Ann. Intern. Med.* 92:45-52.
- Hébert, G. A., A. G. Steigerwalt, and D. J. Brenner. 1980. *Legionella mcdadei* species nova: classification of a third species of *Legionella* associated with human pneumonia. *Curr. Microbiol.* 3:255-257.
- Herwaldt, L. A., G. W. Gorman, T. McGrath, S. Toma, B. Brake, A. W. Hightower, J. Jones, A. L. Reingold, P. A. Boxer, P. W. Tang, C. W. Moss, H. Wilkinson, D. J. Brenner, A. G. Steigerwalt, and C. V. Broome. 1984. A new *Legionella* species, *Legionella feeleyi* species nova, causes Pontiac fever in an automobile plant. *Ann. Intern. Med.* 84:333-338.
- Karr, D. E., W. F. Bibb, and C. W. Moss. 1982. Isoprenoid quinones of the genus *Legionella*. *J. Clin. Microbiol.* 15:1044-1048.
- Kaufmann, A. F., J. E. McDade, C. M. Patton, J. V. Bennett, P. Skaliy, J. C. Feeley, D. C. Anderson, M. E. Potter, V. F.

- Newhouse, M. B. Gregg, and P. S. Brachman. 1981. Pontiac fever: isolation of the etiologic agent (*Legionella pneumophila*) and demonstration of its mode of transmission. *Am. J. Epidemiol.* **114**:337-347.
32. Kodaka, H., A. Y. Amfield, G. L. Lombard, and V. R. Dowell, Jr. 1982. Practical procedure for demonstrating bacterial flagella. *J. Clin. Microbiol.* **16**:948-952.
 33. Lewallen, K. R., R. M. McKinney, D. J. Brenner, C. W. Moss, D. H. Dail, B. M. Thomason, and R. A. Bright. 1979. A newly identified bacterium phenotypically resembling, but genetically distinct from *Legionella pneumophila*: an isolate in a case of pneumonia. *Ann. Intern. Med.* **91**:831-834.
 34. Marmur, J., and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J. Mol. Biol.* **5**:109-118.
 35. McDade, J. E., D. J. Brenner, and F. M. Bozeman. 1979. Legionnaires' disease bacterium isolated in 1947. *Ann. Intern. Med.* **90**:659-661.
 36. McDade, J. E., C. C. Shepard, D. W. Fraser, T. R. Tsai, M. A. Redus, W. R. Dowdle, and the Laboratory Investigation Team. 1977. Legionnaires' disease: isolation of a bacterium and demonstration of its role in other respiratory disease. *N. Engl. J. Med.* **297**:1197-1203.
 37. McKinney, R. M., R. K. Porschen, P. H. Edelstein, M. L. Bissett, P. P. Harris, S. P. Bondell, A. G. Steigerwalt, R. E. Weaver, M. E. Ein, D. S. Linquist, R. S. Kops, and D. J. Brenner. 1981. *Legionella longbeachae* sp. nov., another etiologic agent of human pneumonia. *Ann. Intern. Med.* **94**:739-743.
 38. McKinney, R. M., L. Thacker, P. P. Harris, K. R. Lewallen, G. A. Hébert, P. H. Edelstein, and B. M. Thomason. 1979. Four serogroups of Legionnaires' disease bacteria defined by immunofluorescence. *Ann. Intern. Med.* **90**:621-624.
 39. McKinney, R. M., H. M. Wilkinson, H. W. Sommers, B. J. Fikes, K. R. Sasseville, M. M. Yungbluth, and J. S. Wolf. 1980. *Legionella pneumophila* serogroup six: isolation from cases of legionellosis, identification by immunofluorescence staining, and immunologic response to infection. *J. Clin. Microbiol.* **12**:395-401.
 40. Morris, G. K., C. M. Patton, J. C. Feeley, S. E. Johnson, G. Gorman, W. T. Martin, P. Skaliy, G. F. Mallison, B. D. Politi, and D. C. Mackel. 1979. Isolation of Legionnaires' disease bacterium from environmental samples. *Ann. Intern. Med.* **90**:664-666.
 41. Morris, G. K., A. G. Steigerwalt, J. C. Feeley, E. S. Wong, W. T. Martin, C. M. Patton, and D. J. Brenner. 1980. *Legionella gormanii* sp. nov. *J. Clin. Microbiol.* **12**:718-721.
 42. Moss, C. W. 1981. Gas-liquid chromatography as an analytical tool in microbiology. *J. Chromatogr.* **203**:337-347.
 43. Moss, C. W., W. F. Bibb, D. E. Karr, G. O. Guerrant, and M. A. Lambert. 1983. Cellular fatty acid composition and ubiquinone content of *Legionella feeleyi* sp. nov. *J. Clin. Microbiol.* **18**:917-919.
 44. Moss, C. W., and G. O. Guerrant. 1983. Separation of bacterial ubiquinones using reverse-phase high-performance liquid chromatography. *J. Clin. Microbiol.* **18**:15-17.
 45. Moss, C. W., R. E. Weaver, S. B. Dees, and W. B. Cherry. 1977. Cellular fatty acid composition of isolates from Legionnaires' disease. *J. Clin. Microbiol.* **6**:140-143.
 46. Orrison, L. H., W. B. Cherry, and D. Milan. 1981. Isolation of *Legionella* from cooling tower water by filtration. *Appl. Environ. Microbiol.* **41**:1202-1205.
 47. Orrison, L. H., W. B. Cherry, R. L. Tyndall, C. B. Fliermans, S. B. Gough, M. A. Lambert, W. F. Bibb, L. K. McDougal, and D. J. Brenner. 1983. *Legionella oakridgensis*: an unusual new species isolated from cooling tower water. *Appl. Environ. Microbiol.* **45**:536-545.
 48. Pasculle, A. W., J. C. Feeley, R. J. Gibson, L. G. Cordes, R. L. Myerowitz, C. M. Patton, G. W. Gorman, C. L. Carmack, J. W. Ezzell, and J. N. Dowling. 1980. Pittsburgh pneumonia agent: direct isolation from human lung tissue. *J. Infect. Dis.* **141**:727-732.
 49. Tang, P. W., S. Toma, C. W. Moss, A. G. Steigerwalt, T. G. Colligan, and D. J. Brenner. 1984. *Legionella bozemanii* serogroup 2: a new etiologic agent. *J. Clin. Microbiol.* **19**:30-33.
 50. Tatlock, H. 1944. A rickettsia-like organism recovered from guinea pigs. *Proc. Soc. Exp. Biol. Med.* **57**:95-99.
 51. Thacker, W. L., H. W. Wilkinson, and R. F. Benson. 1983. Comparison of slide agglutination test and direct immunofluorescence assay for identification of *Legionella* isolates. *J. Clin. Microbiol.* **18**:1113-1118.
 52. Thomason, B. M., F. W. Chandler, and D. G. Hollis. 1979. Flagella on Legionnaires' disease bacteria: an interim report. *Ann. Intern. Med.* **91**:224-226.
 53. Thornsberry, C., and L. A. Kirven. 1978. Beta-lactamase of the Legionnaires' bacterium. *Curr. Microbiol.* **1**:51-54.
 54. Weaver, R. E., and J. C. Feeley. 1979. Cultural and biochemical characterization of the Legionnaires' disease bacterium, p. 20-25. In G. L. Jones and G. A. Hébert (ed.), "Legionnaires'," the disease, the bacterium and methodology. Centers for Disease Control, Atlanta.