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Legionella fairfieldensis sp. nov. Isolated from Cooling Tower Waters in Australia

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Three *Legionella*-like organisms were isolated from water from the cooling towers of two Australian institutions. The strains grew on buffered charcoal-yeast extract (BCYE) agar but not on BCYE agar in the absence of L-cysteine. Gas-liquid chromatography profiles of the isolates were consistent with those for *Legionella* spp. They were serologically distinct from other legionellae in a slide agglutination test. DNA hybridization studies showed that the three isolates belong to a new species of *Legionella*, *Legionella fairfieldensis* (ATCC 49588).

Between February and April 1987, three *Legionella*-like organisms were isolated in Fairfield, Victoria, Australia, from the cooling tower waters of two institutions. Water from the air-conditioning units at the institutions is monitored regularly for the presence of *Legionella* strains. In the present report, we describe the growth, physiologic, gas-liquid chromatographic, serologic, and DNA characteristics of three isolates that were found to constitute a new *Legionella* species, *Legionella fairfieldensis*.

MATERIALS AND METHODS

Isolation procedure. Bacteria from 1-liter samples of cooling tower water from the two institutions (one a hospital and the other a scientific institution) were concentrated by filtration through 0.22-μm-pore-size filters (Millipore, Bedford, Mass.). The filters were then placed in approximately 10 ml of water and vortexed to resuspend the bacteria. A portion of each concentrated sample was also heat treated at 50°C for 30 min. The non-heat-treated concentrated samples were inoculated onto antimicrobial agent-supplemented (Oxoid MWY-supplemented) buffered charcoal-yeast extract (BCYE) agar plates (Oxoid U.S.A., Inc., Columbia, Md.). The heat-treated suspensions were inoculated onto BCYE agar plates; onto antimicrobial agent-supplemented BCYE (Oxoid MWY-supplemented) agar plates containing (per 100 ml) 0.3 g of glycine, 5,000 IU of polymyxin B, 8 mg of anisomycin, 100 μg of vancomycin, 1 mg of bromothymol blue, and 1 mg of bromocresol purple; and onto Oxoid BMPAα agar plates containing (per 100 ml) 400 μg of cefamandole, 8,000 IU of polymyxin B, and 8 mg of anisomycin. All plates were incubated at 37°C in 5% CO_2 for 7 days. Suspected legionellae were picked after 3 to 7 days of growth.

Three *Legionella*-like isolates (1725-AUS-E, 1732-AUS-E, and 1733-AUS-E) were obtained from the water specimens of the cooling towers. These isolates could not be identified as belonging to any of the previously described *Legionella* species. They were forwarded through Fairfield Hospital, Fairfield, Victoria, Australia, to the Centers for Disease Control for further study.

Growth and biochemical tests. At the Centers for Disease Control, the isolates were grown on BCYE agar for use in all but two tests. The buffer was omitted for determination of autofluorescence, and cysteine was omitted for determination of the cysteine requirement (14). Physiologic tests for catalase, gelatinase, oxidase, urease, β-lactamase, hippurate hydrolysis, nitrate reduction, glucose fermentation, flagella, autofluorescence, and browning of tyrosine-supplemented agar were done as described previously (7).

Cellular fatty acids. Four batches per strain of cells of strains 1725-AUS-E, 1732-AUS-E, and 1733-AUS-E were grown on BCYE agar and analyzed for cellular nonhydroxy, monohydroxy, and dihydroxy fatty acids by gas-liquid chromatography after sequential alkaline saponification and acid hydrolysis to effect fatty acid liberation (9, 10). Fatty acid profiles, adjusted for relative molar response of the individual components, were calculated on a relative abundance basis, with the level of the most abundant fatty acid in each class considered equal to 100. The class moles percent of each of the individual components was also calculated (Table 1).

Slide agglutination test. The three isolates were tested with antisera to all previously characterized *Legionella* species ($n = 30$) and serogroups ($n = 49$) (1, 2, 4, 6, 12, 14, 15). Antisera to the three isolates, 1725-AUS-E, 1732-AUS-E, and 1733-AUS-E, were prepared and tested by slide agglutination as described previously (13).

DNA studies. The guanine-plus-cytosine (G+C) content of DNA from each isolate was determined spectrophotometrically by thermal denaturation (8). DNA hybridization was done by the hydroxyapatite method at 60 and 75°C (5, 16). Unlabeled DNA was prepared from all *Legionella* species except *L. anisa*, *L. rubrilucens*, *L. cherrii*, *L. santicrucis*, and *L. steigerwaltii*. These five species show 50% or more relatedness to at least one species that was tested. The strains used are indicated in Table 2. Since none of the test strains were ≥50% related to any described *Legionella* species, it was not necessary to test the five omitted species.

RESULTS

Growth characteristics and biochemical tests. The three *Legionella*-like isolates were typical of *Legionella* species in that they required cysteine for growth. They were gram-

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TABLE 1. Nonhydroxy, monohydroxy, and dihydroxy fatty acid profiles of strains of *L. fairfieldensis*, expressed as relative molar abundance in class and moles percent in class

Fatty acid[a]	Relative abundance (mol%) of strain:		
	1725-AUS-E	1732-AUS-E	1733-AUS-E
Nonhydroxy (approx 85 mol% of total)			
a-$C_{13:0}$	0 (0)	2 (1)	3 (1)
i-$C_{14:0}$	14 (5)	8 (2)	12 (3)
n-$C_{14:0}$	2 (1)	3 (1)	2 (1)
a-$C_{15:0}$	15 (5)	84 (21)	82 (20)
n-$C_{15:1}$	6 (2)	1 (0)	3 (1)
n-$C_{15:0}$	5 (2)	2 (0)	2 (0)
i-$C_{16:0}$	100 (36)	63 (16)	79 (20)
n-$C_{16:1}$	51 (18)	100 (25)	100 (25)
n-$C_{16:0}$	33 (12)	68 (17)	48 (12)
a-$C_{17:0}$	9 (3)	43 (11)	43 (11)
cyc17	27 (10)	12 (3)	17 (4)
n-$C_{18:0}$	4 (1)	5 (1)	5 (1)
n-$C_{20:0}$	8 (3)	1 (0)	2 (1)
3-Monohydroxy (approx 10 mol% of total)			
i-C_{14}h	79 (21)	35 (10)	46 (12)
n-C_{14}h	100 (26)	100 (29)	100 (26)
i-C_{15}h	1 (0)	1 (0)	1 (0)
a-C_{15}h	9 (2)	28 (8)	31 (8)
n-C_{15}h	7 (2)	2 (1)	3 (1)
i-C_{16}h	6 (2)	3 (1)	5 (1)
n-C_{16}h	11 (3)	55 (16)	50 (13)
i-C_{18}h	3 (1)	2 (1)	5 (1)
n-C_{18}h	16 (4)	45 (13)	44 (11)
a-C_{19}h	1 (0)	8 (2)	11 (3)
n-C_{19}h	6 (1)	2 (1)	4 (1)
i-C_{20}h	5 (1)	2 (0)	4 (1)
n-C_{20}h	93 (24)	50 (15)	70 (18)
a-C_{21}h	1 (0)	3 (1)	6 (1)
n-C_{21}h	16 (4)	1 (0)	2 (1)
i-C_{22}h	4 (1)	0 (0)	1 (0)
n-C_{22}h	20 (5)	1 (0)	3 (1)
2,3-Dihydroxy (approx 5 mol% of total)			
n-C_{12}h2	5 (2)	4 (2)	5 (2)
a-C_{13}h2	0 (0)	3 (1)	3 (2)
n-C_{13}h2	5 (2)	2 (1)	2 (1)
i-C_{14}h2	92 (41)	42 (22)	64 (29)
n-C_{14}h2	100 (3)	100 (53)	100 (46)
a-C_{15}h2	8 (3)	29 (15)	35 (16)
n-C_{15}h2	5 (2)	1 (1)	1 (1)
i-C_{16}h2	3 (2)	4 (2)	5 (2)
n-C_{16}h2	5 (2)	5 (3)	5 (2)

[a] Abbreviations: i, isobranched; a, anteisobranched; n, normal (straight chain); cyc, cyclopropane; h, monohydroxy fatty acid; h2, dihydroxy fatty acid. The number following the colon indicates the number of double bonds. The monohydroxy acids are 3-hydroxylated and saturated; the dihydroxy acids are 2,3-hydroxylated and saturated. Trace components (relative abundance, ≤2 in all strains) were n-$C_{14:1}$, i-$C_{15:0}$, i-$C_{17:0}$, n-$C_{17:0}$, and i-$C_{18:0}$ nonhydroxy fatty acids and n-C_{12}h, n-C_{13}h, i-C_{15}h, a-C_{17}h, and n-C_{17}h monohydroxy fatty acids.

negative rods with single polar flagella. No autofluorescence was observed when the isolates were exposed to long-wave (365-nm) UV light. Physiologic test results were negative for reduction of nitrate to nitrite, urease, acid production from D-glucose, browning of tyrosine-supplemented agar, gelatinase, hydrolysis of hippurate, and β-lactamase. They were positive in reactions for catalase and weakly positive for oxidase.

Cellular fatty acid analysis. The nonhydroxy, monohydroxy, and dihydroxy fatty acid profiles of strains 1725-AUS-E, 1732-AUS-E, and 1733-AUS-E are shown in Table 1. Branched- and straight-chain saturated and unsaturated nonhydroxy fatty acids represented approximately 85 mol% of the total fatty acids, while saturated 3-hydroxy fatty acids and 2,3-dihydroxy fatty acids, in a ratio of approximately 2:1, made up the remaining 15 mol%.

Slide agglutination test. Strains 1725-AUS-E, 1732-AUS-E, and 1733-AUS-E did not agglutinate with antisera prepared to any of the 30 previously described *Legionella* species or 49 serogroups. Antisera prepared against the isolates reacted 4+ with all three isolates and did not agglutinate strains representing any other *Legionella* species or serogroup. Therefore, the three isolates make up a single serogroup.

DNA relatedness studies. DNA relatedness values are given in Table 2. Labeled DNA from 1725-AUS-E was 94% related to DNA from 1732-AUS-E and 87% related to DNA from 1733-AUS-E in reactions at 60°C. The percent diver-

TABLE 2. DNA relatedness of *L. fairfieldensis* 1725-AUS-E to other legionellae

Source of unlabeled DNA[a]	% Relatedness to labeled DNA from *L. fairfieldensis* 1725-AUS-E at[b]:	
	60°C (% divergence)	75°C
L. fairfieldensis 1725-AUS-E	100 (0.0)	100
L. fairfieldensis 1732-AUS-E	94 (0.0)	100
L. fairfieldensis 1923-ENG-E[c]	89 (0.5)	93
L. fairfieldensis 1733-AUS-E	87 (0.5)	89
L. jamestownensis JA-26-G1-E2	17	
L. micdadei TATLOCK	15	
L. maceachernii PX-1-G2-E2	15	
L. feeleii WO-44C-C3	14	
L. jordanis BL-540	13	
L. erythra SE-32A-C8	13	
L. hackeliae Lansing 2	13	
L. bozemanii WIGA	12	
L. spiritensis Mount Saint Helens 9	12	
L. pneumophila Philadelphia 1	11	
L. dumoffii NY-23	11	
L. gormanii LS-13	11	
L. parisiensis PF-209C-C2	10	
"*L. quateirensis*"[d] 1335	10	
L. brunensis 441-1	10	
L. longbeachae Long Beach 4	9	
"*L. nautarum*"[d] 1224	9	
L. tucsonensis 1087-AZ-H	9	
L. oakridgensis Oak Ridge 10	8	
L. israelensis Bercovier 4	8	
L. moravica 316-36	8	
L. sainthelensi Mount Saint Helens 4	7	
"*L. worsleiensis*"[d] 1347	7	
L. gratiana Lyon 8420412	7	
L. birminghamenesis 1407-AL-H	6	
"*L. londiniensis*"[d] 1477	5	
L. wadsworthii 81-716A	4	
L. cincinnatiensis 72-OH-H	4	
L. quinlivanii 1448-AUS-E	3	
"*L. geestiana*"[d] 1308	1	

[a] *L. fairfieldensis* 1725-AUS-E and the strains of all other species listed except *L. quinlivanii* are type strains. *L. quinlivanii* 1448-AUS-E is 100% related to the type strain, 1442-AUS-E.

[b] All experiments were done at least twice. A blank space indicates that the reaction was not done.

[c] *L. fairfieldensis* 1923-ENG-E was labeled in these reactions.

[d] Names in quotation marks have not been validly described, but the strains listed are the putative type strains for five additional *Legionella* species.

gence in these reaction was 0 and 0.5, respectively. The relatedness values in reactions at 75°C were 100 and 89%, respectively. Strain 1725-AUS-E was 17% or less related to the 30 other *Legionella* species tested.

DISCUSSION

Isolates 1725-AUS-E, 1732-AUS-E, and 1733-AUS-E were presumptively identified as *Legionella* strains by their tinctorial, morphologic, and growth characteristics. This identification was confirmed by the presence of predominantly branched-chain cellular fatty acids in the isolates. It can be seen in Table 1 that, with respect to fatty acid profiles, strain 1725-AUS-E differs somewhat from 1732-AUS-E and 1733-AUS-E, which resemble each other closely. The unifying "signature" compounds in this group are the 2,3-dihydroxy fatty acids. The pattern of n-C_{14}h2 at the highest concentration followed by i-C_{14}h2 is reflective of the pattern of these chain lengths in the monohydroxy fatty acid profiles. This pattern differs from those of the other dihydroxy fatty acid-containing *Legionella* species, *L. israelensis*, *L. maceachernii*, *L. micdadei*, and the three subspecies of *L. pneumophila*, in which i-C_{14}h2 is the dominant dihydroxylated component (9–11).

The high DNA relatedness observed between strain 1725-AUS-E and the other two isolates indicated that these isolates represent one or more strains of a new *Legionella* species, *L. fairfieldensis*. In addition, the isolates were shown by the slide agglutination test to represent a single serogroup within the *L. fairfieldensis* species.

Whether *L. fairfieldensis* will be recognized as a human pathogen remains to be determined. In a previous report (3), results of a serum survey from one of the institutions, the hospital, indicated that none of 68 patients or 33 staff members demonstrated antibodies to the *L. fairfieldensis* strain isolated at that location.

Description of *Legionella fairfieldensis* sp. nov. *Legionella fairfieldensis* (fair.field.en'sis. N. L. Fem. adj. *fairfieldensis* coming from Fairfield, Victoria, Australia) is a gram-negative rod with a single polar flagellum. *L. fairfieldensis* grows on BCYE agar but not on media lacking L-cysteine. Its cellular fatty acids are predominantly branch-chained. It is negative in tests for reduction of nitrate to nitrite, urease, acid production from D-glucose, browning of tyrosine-supplemented agar, gelatinase, hydrolysis of hippurate, and β-lactamase. It is positive in reactions for catalase and weakly positive for oxidase. It is serologically distinct from other described legionellae and can be identified by the slide agglutination test. The type strain of *L. fairfieldensis* is 1725-AUS-E (ATCC 49588), which has a G+C content of 42 mol%. It was isolated from cooling tower water in Fairfield, Victoria, Australia, in February 1987.

After the conclusion of this study a fourth strain, 1923-ENG-E, isolated from a cooling tower water specimen in England was shown to belong in the *L. fairfieldensis* species. Labeled DNA from strain 1923-ENG-E was 89% related to 1725-AUS-E at 60°C, with 0.5% divergence in the related sequences, and was 93% related to 1725-AUS-E at 75°C. Strain 1923-ENG-E was in the same serogroup as the Australian isolates.

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REFERENCES

1. **Benson, R. F., W. L. Thacker, F. C. Fang, B. Kanter, W. R. Mayberry, and D. J. Brenner.** 1990. *Legionella sainthelensi* serogroup 2 isolated from patients with pneumonia. Res. Microbiol. **141**:453–463.
2. **Benson, R. F., W. L. Thacker, R. P. Walters, P. A. Quinlivan, W. R. Mayberry, D. J. Brenner, and H. W. Wilkinson.** 1989. *Legionella quinlivanii* sp. nov.: a new species of *Legionella* isolated from water. Curr. Microbiol. **18**:195–197.
3. **Bettelheim, K. A., L. Hawes, R. J. Warren, and B. W. Dwyer.** 1988. Studies after the isolation of a *Legionella*-like organism from the air-conditioning system of two wards of a hospital. Med. J. Aust. **148**:159–160.
4. **Bornstein, N., D. Marmet, M. Surgot, M. Nowicki, H. Meugnier, J. Fleurette, E. Ageron, F. Grimont, P. A. D. Grimont, W. L. Thacker, R. F. Benson, and D. J. Brenner.** 1989. *Legionella gratiana* sp. nov. isolated from French spa water. Res. Microbiol. **140**:541–552.
5. **Brenner, D. J., A. C. McWhorter, J. K. L. Knutson, and A. G. Steigerwalt.** 1982. *Escherichia vulneris*: a new species of *Enterobacteriaceae* associated with human wounds. J. Clin. Micro-

biol. **15:**1133–1140.

6. **Brenner, D. J., A. G. Steigerwalt, P. Epple, W. F. Bibb, R. M. McKinney, R. W. Starnes, J. M. Colville, R. K. Selander, P. H. Edelstein, and C. W. Moss.** 1988. *Legionella pneumophila* serogroup Lansing 3 isolated from a patient with fatal pneumonia, and descriptions of *L. pneumophila* subsp. *pneumophila* subsp. nov., *L. pneumophila* subsp. *fraseri* subsp. nov., and *L. pneumophila* subsp. *pascullei* subsp. nov. J. Clin. Microbiol. **26:** 1695–1703.
7. **Brenner, D. J., A. G. Steigerwalt, G. W. Gorman, H. W. Wilkinson, W. F. Bibb, M. Hackel, R. L. Tyndall, J. Campbell, J. C. Feeley, W. L. Thacker, P. Skaliy, W. T. Martin, B. J. Brake, B. S. Fields, H. V. McEachern, and L. K. Corcoran.** 1985. Ten new species of *Legionella*. Int. J. Syst. Bacteriol. **35:**50–59.
8. **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.
9. **Mayberry, W. R.** 1981. Dihydroxy and monohydroxy fatty acids in *Legionella pneumophila*. J. Bacteriol. **147:**373–381.
10. **Mayberry, W. R.** 1984. Monohydroxy and dihydroxy fatty acid composition of *Legionella* species. Int. J. Syst. Bacteriol. **34:**321–326.
11. **Mayberry, W. R.** Unpublished data.
12. **Thacker, W. L., R. F. Benson, R. B. Schifman, E. Pugh, A. G. Steigerwalt, W. R. Mayberry, D. J. Brenner, and H. W. Wilkinson.** 1989. *Legionella tucsonensis* sp. nov. isolated from a renal transplant recipient. J. Clin. Microbiol. **27:**1831–1834.
13. **Thacker, W. L., B. B. Plikaytis, and H. W. Wilkinson.** 1985. Identification of 22 *Legionella* species and 33 serogroups with the slide agglutination test. J. Clin. Microbiol. **21:**779–782.
14. **Wilkinson, H. W.** 1988. Hospital-laboratory diagnosis of *Legionella* infections, revised ed. Centers for Disease Control, Atlanta.
15. **Wilkinson, H. W., V. Drasar, W. L. Thacker, R. F. Benson, J. Schindler, B. Potuznikova, W. R. Mayberry, and D. J. Brenner.** 1988. *Legionella moravica* sp. nov. and *Legionella brunensis* sp. nov. isolated from cooling-tower water. Ann. Inst. Pasteur/ Microbiol. (Paris) **139:**393–402.
16. **Wilkinson, H. W., W. L. Thacker, R. F. Benson, S. S. Polt, E. Brookings, W. R. Mayberry, D. J. Brenner, R. G. Gilley, and J. K. Kirklin.** 1987. *Legionella birminghamensis* sp. nov. isolated from a cardiac transplant recipient. J. Clin. Microbiol. **25:**2120–2122.