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Legionella adelaidensis, a New Species Isolated from Cooling Tower Water

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A *Legionella*-like organism (strain 1762-AUS-E) was isolated from a cooling tower of an air-conditioning system in Adelaide, South Australia, Australia. The isolate was presumptively identified as a *Legionella* strain by its growth requirement for L-cysteine and its cellular branched-chain fatty acids. Strain 1762-AUS-E was serologically distinct in the slide agglutination test with absorbed antisera. DNA hybridization confirmed that it is a new *Legionella* species for which the name *Legionella adelaidensis* is proposed.

Legionella species are ubiquitous freshwater inhabitants (7) which occasionally infect susceptible humans. Since the initial isolation of *Legionella pneumophila* in 1976, 31 species (3, 4, 17, 19, 20) have been described, consisting of 50 serogroups based on agglutinating surface antigens. Sixteen of these species are recognized as pathogenic (16, 17, 19). The remaining 15 species have been isolated only from environmental sources.

Outbreaks of Legionnaires disease have been associated with cooling towers and evaporative condensers (1, 8), potable water sources, and contaminated humidifiers and nebulizers (2, 9). In epidemics, aerosolized spray from cooling towers has been implicated in several cases as the mode of transmission for *Legionella* species.

In 1987, a *Legionella*-like organism that could not be identified with available reagents was isolated during routine sampling of a cooling tower in Adelaide, South Australia, Australia. In this report, we show that this *Legionella*-like organism is a new species of *Legionella*, *L. adelaidensis*.

MATERIALS AND METHODS

Isolation procedure. The isolate was grown from cooling tower water and submitted to the Institute of Medical and Veterinary Science, Adelaide, for routine identification as a *Legionella* species. The water sample was examined as described previously (21) and inoculated onto buffered charcoal-yeast extract (BCYE) agar containing BMPAα selective supplement (Oxoid Ltd., Basingstoke, United Kingdom).

Growth and biochemical tests. Strain 1762-AUS-E was grown on BCYE agar and inoculated onto BCYE agar without added cysteine and charcoal-yeast extract without the ACES buffer for testing for the cysteine requirement and autofluorescence, respectively (19). Methods used for characterizing *Legionella*-like organisms by physiologic tests for catalase, gelatinase, oxidase, urease, β-lactamase, hippurate hydrolysis, nitrate reduction, acid production from D-glucose, flagella, autofluorescence, and browning of tyrosine-supplemented media have been described previously (6, 19).

Slide agglutination test. Strain 1762-AUS-E was tested with antisera to all previously named and published *Legionella* species ($n = 31$) and serogroups ($n = 50$) (3, 4, 6, 17, 19, 20). Antiserum to strain 1762-AUS-E was prepared and tested as described previously (18).

Direct fluorescent antibody. Testing of strain 1762-AUS-E with conjugates provided by the Centers for Disease Control for *L. bozemanii* serogroup 1, *L. dumoffii*, *L. gormanii*, *L. jordanis*, *L. oakridgensis*, *L. micdadei*, and *L. longbeachae* serogroups 1 and 2 was performed at the Institute of Medical and Veterinary Science.

Latex agglutination. Strain 1762-AUS-E was tested with latex agglutination reagents for *L. anisa*, *L. erythra*, *L. rubrilucens*, *L. jamestowniensis*, *L. quinlivanii*, *L. longbeachae* serogroup 1, *L. cincinnatiensis*, and *L. pneumophila* serogroups 1 to 14 (21).

Readily extractable cellular fatty acids. The isolate was grown for 3 to 4 days on BCYE agar plates at 35°C. Organisms were removed from the surface of one or two plates, depending on the density of growth, and suspended in 1 ml of water. Cellular fatty acids were extracted and analyzed as described previously (21). Chromatograms were recorded and analyzed by using Nelson Analytical series 3000 Chromatography Data System software. Fatty acid peaks were identified by comparison of relative retention times with those of a standard bacterial fatty acid mixture (product no. 4-7080; Supelco, Bellefonte, Pa.). Peak identities were confirmed by mass spectrometry when necessary.

Total cellular fatty acids. By using two different methods for liberating fatty acids, 20 separate batches (five plates per batch) of strain 1762-AUS-E were analyzed for total cellular nonhydroxy, monohydroxy, and dihydroxy fatty acids by gas-liquid chromatography. Fatty acid esters were identified by comparison of equivalent chain length values with those of commercial standards or previously characterized components (13, 14). Fatty acid profiles were adjusted for relative molar response of each component and calculated with the most abundant fatty acid in each class considered to be equal to 100.

Ubiquinone analysis. Ubiquinones were extracted by a modification of the method of Karr et al. (10) and analyzed as described previously (21).

DNA studies. The guanine-plus-cytosine content of DNA was determined spectrophotometrically by thermal denaturation (12). The method for the preparation of unlabeled and $^{32}PO_4$ in vitro-labeled DNA has been described previously,

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as has the determination of DNA relatedness by the hydroxyapatite method at 60°C (5, 6).

RESULTS

Growth and biochemical tests. Strain 1762-AUS-E is a gram-negative rod with a single polar flagellum. The strain grew on BCYE agar, but not on BCYE agar without cysteine or on blood agar. Results of physiologic tests were positive for catalase and gelatin liquefaction and negative for nitrate reduction, oxidase, urease, acid production from glucose, hippurate hydrolysis, β-lactamase, autofluorescence, and brown pigment production on tyrosine-supplemented agar.

Serologic testing. Strain 1762-AUS-E reacted weakly (1+) with unabsorbed antiserum to *L. hackeliae* serogroup 1 and *L. dumoffii*. Antiserum prepared against strain 1762-AUS-E at the optimal working dilution of 1:16 gave a 4+ agglutination reaction with its homologous antigen and was negative with all other *Legionella* antigens.

Strain 1762-AUS-E was negative with all direct fluorescent-antibody conjugates tested. No reaction was observed in the latex agglutination test with strain 1762-AUS-E.

Readily extractable cellular fatty acids. Gas-liquid chromatographic analysis of the readily extractable fatty acids of strain 1762-AUS-E showed a pattern of branched- and straight-chain nonhydroxy fatty acids dominated by n-$C_{16:1}$, n-$C_{16:0}$, and n-$C_{20:0}$, with significant concentrations of i-$C_{16:0}$, a-$C_{15:0}$, a-$C_{17:0}$, n-$C_{18:0}$, n-$C_{14:0}$, n-$C_{15:0}$, and n-$C_{15:1}$.

Total cellular fatty acids. Gas-liquid chromatographic analysis of the total cellular fatty acids of strain 1762-AUS-E showed the presence of nonhydroxy and 3-hydroxy fatty acids in a molar ratio of approximately 9:1. No 2,3-dihydroxy fatty acids were found. The fatty acids detected, their relative abundance, and their class mole percent composition are given in Table 1.

Ubiquinone analysis. The ubiquinone composition of strain 1762-AUS-E, expressed as relative abundance and percent composition, is listed in Table 2.

DNA relatedness studies. $^{32}PO_4$-labeled DNA from strain 1762-AUS-E was hybridized with unlabeled DNAs from type strains of 28 *Legionella* species. The three species not tested were related 50% or more to the species that were tested. Relatedness in all cases was 31% or less.

TABLE 1. Major fatty acids of *L. adelaidensis*[a]

Fatty acid	Relative abundance	Class mol%
Nonhydroxy (approx 90% of total)		
i-$C_{14:0}$	7	2
n-$C_{14:1}$	4	1
n-$C_{14:0}$	12	3
i-$C_{15:0}$	6	2
a-$C_{15:0}$	48	12
n-$C_{15:1}$	9	2
n-$C_{15:0}$	16	4
i-$C_{16:0}$	44	11
n-$C_{16:1}$	100	26
n-$C_{16:0}$	92	24
i-$C_{17:0}$	3	1
a-$C_{17:0}$	14	3
n-$C_{17:0}$	3	1
n-$C_{18:0}$	12	3
n-$C_{20:0}$	12	3
3-Hydroxy (approx 10% of total)		
n-C_{12h}	13	4
n-C_{13h}	3	1
i-C_{14h}	100	30
n-C_{14h}	64	19
a-C_{15h}	18	5
i-C_{16h}	3	1
n-C_{16h}	11	3
n-C_{18h}	13	4
n-C_{19h}	3	1
n-C_{20h}	88	27
n-C_{21h}	4	1
n-C_{22h}	4	1

[a] Minor components (relative abundance, <2) include the nonhydroxy acids cyc16, cyc17, i-$C_{18:0}$, n-$C_{19:0}$, n-$C_{22:0}$, and n-$C_{23:0}$, as well as the 3-hydroxy acids n-C_{15h}, n-C_{17h}, i-C_{20h}, and a-C_{21h}. The letters preceeding C indicate the chain configurations: i, iso-branched; a, anteiso-branched; n, normal (straight-chain); cyc, cyclopropane; h, monohydroxy fatty acid. The number following the colon indicates the number of double bonds in unsaturated components.

DISCUSSION

Strain 1762-AUS-E was presumptively identified as a *Legionella* species by its morphologic and growth characteristics. This identification was confirmed by the presence of branched-chain cellular fatty acids. The pattern of n-$C_{16:1}$ and i-C_{14h} as the most abundant nonhydroxy and monohydroxy fatty acids, respectively, is shared by *L. feeleii* and *L. moravica*. The nonhydroxy and monohydroxy fatty acid profiles of these two species and that of *L. adelaidensis* can be readily distinguished (13, 15, 20). The ubiquinone profile, dominated by Q11, is unlike any of the profiles reported for 23 *Legionella* species by Lambert and Moss (11) and appears to represent a new ubiquinone group. DNA hybridization studies confirmed that strain 1762-AUS-E is a new *Legionella* species, with little relatedness to previously described species. There were no serologic cross-reactions observed with antiserum produced against *L. adelaidensis* and the other 31 *Legionella* species. Cross-reaction between *L. adelaidensis* and *L. hackeliae* serogroup 1 and *L. dumoffii* antiserum were not observed when appropriately absorbed antiserum was tested. *L. adelaidensis* is the 32nd species and the 51st serogroup described in the genus *Legionella*.

Description of *L. adelaidensis* sp. nov. *L. adelaidensis* (a. del. aid. en'sis N.L. fem. adj. *adelaidensis*, coming from Adelaide, Australia) is a gram-negative rod with a single polar flagellum. Its cellular fatty acids are predominantly branch chained. It is positive in reactions for catalase and gelatin liquefaction and requires cysteine for growth. It is negative for nitrate reduction, oxidase, urease, hippurate hydrolysis, β-lactamase, autofluorescence, and acid production from D-glucose. Antiserum prepared against strain 1762-AUS-E is negative in the slide agglutination test against all heterologous species and serogroups. The type strain of *L. adelaidensis* is 1762-AUS-E (ATCC 49625). It has a guanine-plus-cytosine content of 40 mol%. It was isolated from cooling tower water.

TABLE 2. Ubiquinone composition of *L. adelaidensis*

Ubiquinone	Relative abundance	% Composition
Q9	2	1
Q10	40	25
Q11	100	64
Q12	15	9

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