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NOTES

Characterization of a *Legionella anisa* Strain Isolated from a Patient with Pneumonia

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***Legionella anisa*, previously found only in environmental specimens, was isolated from a bronchial lavage specimen of an immunocompromised patient with pneumonia. Growth, physiologic, gas-liquid chromatographic, serologic, and DNA characteristics were consistent with those of the type strain of *L. anisa*, WA-316-C3 (ATCC 35292).**

The type strain of *Legionella anisa* (WA-316-C3) was isolated from hospital tap water during an outbreak of nosocomial legionellosis (6). It was not, however, associated with human disease. Until recently, we and others (3) had identified numerous *L. anisa* isolates from environmental sources but not from clinical material. In this report, we describe the characterization of a *L. anisa* strain isolated from a bronchial lavage specimen of a patient with pneumonia.

Case report. The patient was a 65-year-old female with a history of non-insulin-dependent diabetes mellitus of a duration of 5 years and a previous coronary artery bypass graft in 1981. On 7 July 1986 she had a resection of a Dukes stage C carcinoma of the colon. The patient began to cough blood during the week following the operation and was initially treated for an infected pulmonary infarction. The hemoptysis continued, and at bronchoscopy, blood was seen coming from the anterior segment of the left upper lobe. A computerized tomography scan of the chest on 12 August 1986 showed an opacity extending out from the left wall of the left atrium and around the ventricle, which contained some irregular infarction. A lung scan showed that this segment was neither ventilated nor perfused. Cultures of her bronchoalveolar lavage specimens on charcoal yeast extract agar yielded a *Legionella*-like organism after 72 h. Gram staining showed poorly staining gram-negative rods with long filamentous forms. The isolate was oxidase negative and catalase positive. There was no growth on cysteine-free charcoal yeast extract agar. Illumination with long-wave (365-nm) UV light produced bright blue-white autofluorescence. Immunofluorescence staining for *Legionella pneumophila* was negative. The patient was treated with erythromycin; however, episodic hemoptysis persisted until December 1986, when she had a lingulectomy. The excised lung was reported as showing focal interstitial lymphoid infiltrates. This is an end-stage pattern and most probably represented an organized pneumonia. No *Legionella* organisms were cultured from the lung tissue.

The *Legionella* isolate obtained from the bronchial lavage specimen was forwarded through Fairfield Hospital, Fairfield, Victoria, Australia, to the Centers for Disease Control for reference identification. At the Centers for Disease Control, a single transferred colony of the *Legionella* isolate (1497-AUS-H) grew aerobically on buffered-charcoal yeast extract agar (11) but not on buffered-charcoal yeast extract agar without cysteine or on blood agar (BBL Microbiology Systems). The organisms were gram-negative rods with single polar flagella that showed blue-white autofluorescence when exposed to long-wave (365-nm) UV light. Most strains of *L. anisa*, including the type strain, WA-316-C3, autofluoresce blue-white under UV light. However, some strains of *L. anisa* (6), confirmed by DNA hybridization, are known to be nonfluorescent.

The slide agglutination test (10) was used to serologically identify strain 1497-AUS-H. Strain 1497-AUS-H gave a reaction of 4+ in the slide agglutination test with unabsorbed antisera to *Legionella micdadei*, *Legionella bozemanii* serogroups 1 and 2, and *L. anisa*. The only reaction observed with serogroup-specific (absorbed) antisera was 3+ with *L. anisa* antiserum. Physiologic test (5) results for strains 1497-AUS-H and WA-316-C3 were negative for urease, nitrate reduction, glucose fermentation, and hippurate hydrolysis. Positive reactions were observed for catalase, gelatinase, oxidase, and β-lactamase. Strain WA-316-C3 showed browning of tyrosine-supplemented agar, whereas strain 1497-AUS-H showed no change.

At least six separate batches each of strains WA-316-C3 and 1497-AUS-H were analyzed by gas-liquid chromatography for cellular nonhydroxy, monohydroxy, and dihydroxy fatty acids; two different methods for liberation of fatty acids (7, 8) were used. Fatty acid profiles were adjusted for relative molar response of each component and calculated as relative abundance, with the most abundant component in each class considered equal to 100. The moles percent of each component within each class was also calculated (Table 1). Both strains showed nonhydroxy and 3-hydroxy fatty acids in an approximate molar ratio of 9:1. Dihydroxy fatty acids were not detected. Although the pattern of a-$C_{15:0}$ as the most abundant nonhydroxy fatty acid is relatively common among species of *Legionella*, particularly among the

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TABLE 1. Fatty acid profiles of *L. anisa* WA-316-C3 and 1497-AUS-H

Fatty acid[a]	Relative abundance (mol%) of strain:	
	WA-316-C3	1497-AUS-H
Nonhydroxy		
i-$C_{14:0}$	19 (6)	31 (9)
n-$C_{14:0}$	1 (0)	1 (0)
i-$C_{15:0}$	1 (0)	2 (1)
a-$C_{15:0}$	100 (33)	100 (30)
n-$C_{15:1}$	5 (2)	5 (1)
n-$C_{15:0}$	5 (2)	7 (2)
i-$C_{16:0}$	67 (22)	79 (24)
n-$C_{16:1}$	28 (9)	17 (5)
cyc 16	3 (1)	6 (2)
n-$C_{16:0}$	17 (6)	24 (7)
i-$C_{17:0}$	2 (1)	3 (1)
a-$C_{17:0}$	24 (8)	16 (5)
cyc 17	24 (8)	35 (10)
n-$C_{17:0}$	4 (1)	5 (1)
n-$C_{18:0}$	2 (1)	2 (1)
n-$C_{19:0}$	1 (0)	2 (1)
3-Hydroxy		
n-C_{13}h	2 (0)	1 (0)
i-C_{14}h	11 (2)	10 (3)
n-C_{14}h	38 (8)	28 (7)
i-C_{15}h	1 (0)	1 (0)
a-C_{15}h	17 (4)	18 (5)
n-C_{15}h	31 (6)	37 (9)
i-C_{16}h	100 (21)	100 (26)
n-C_{16}h	88 (18)	59 (15)
i-C_{17}h	10 (2)	13 (3)
a-C_{17}h	29 (6)	22 (6)
n-C_{17}h	32 (7)	22 (6)
i-C_{18}h	8 (2)	5 (1)
n-C_{18}h	62 (13)	39 (10)
a-C_{19}h	3 (1)	2 (1)
n-C_{19}h	24 (5)	14 (4)
n-C_{20}h	24 (5)	18 (5)
n-C_{21}h	2 (0)	3 (1)

[a] The letter preceding C indicates the configuration of the chain. Abbreviations: i, iso-branched; a, anteiso-branched; n, normal (straight chain); cyc, cyclopropane; h, monohydroxy fatty acid.

blue-white autofluorescent species, the combination of a-$C_{15:0}$ and i-C_{16}h as the most abundant nonhydroxy and monohydroxy acids, respectively, is not found in any other autofluorescent species examined to date and appears to be a characteristic of *L. anisa*.

DNA hybridization reactions identified strain 1497-AUS-H as *L. anisa* by the hydroxyapatite method (5). Strain 1497-AUS-H was >90% related to *L. anisa* type strain WA-316-C3 at both optimal (60°C) and stringent (75°C) temperatures for DNA reassociation.

Twenty-nine species (1, 4, 9, 11, 12) and 47 serogroups based on agglutinating surface antigens have been described in the genus *Legionella*. Fifteen of these species, comprising 33 serogroups, have been reported as pathogenic for humans. These results show that *L. anisa*, previously isolated only from the environment, can also be isolated from clinical specimens. Further studies are needed to define its significance as a human pathogen.

Since this paper was submitted for publication, Bornstein et al. (2) have reported on a human pleural infection caused by *L. anisa*. *L. anisa* was isolated from the pleural fluid of an immunosuppressed patient with a severe respiratory infection.

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