

## Cellular Fatty Acid Composition of *Lautropia mirabilis*

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Ten strains of *Lautropia mirabilis* (ATCC 51599<sup>T</sup> and nine phenotypically similar clinical isolates) were examined for cellular fatty acid (CFA) composition to evaluate their chemical relatedness to known bacterial species and groups. The CFAs were liberated from whole cells by base hydrolysis, methylated, and analyzed by gas-liquid chromatography. CFA profiles were generated by using a commercial software package (MIDI, Newark, Del.). All strains tested had an identical CFA profile characterized by major amounts of 16:1 $\omega$ 7c (41%) and 16:0 (44%); smaller amounts (1 to 4%) of 3-OH-10:0, 12:0, 14:0, 15:0, and 18:1  $\omega$ 7c; trace amounts (<1%) of 10:0, 18:2 and 18:0; and no cyclopropane acids. This profile was similar to the CFA profiles of *Acidovorax delafieldii*, *Comamonas terrigena*, and strains of an unclassified Centers for Disease Control group designated weak oxidizer group 1. CFA analysis, when supplemented by phenotypic characterization, is useful for the identification of *L. mirabilis* isolates.

*Lautropia mirabilis* was first described by Gerner-Smidt et al. in 1994 (2). This species is a motile facultatively anaerobic gram-negative coccus which ferments glucose, fructose, sucrose, and mannitol; reduces nitrate and nitrite; and produces positive reactions for oxidase, urease, and, sometimes weakly, catalase. *L. mirabilis* displays an extremely polymorphic cell morphology with diameters ranging from with 1 to >10  $\mu$ m. Phylogenetic characterization based on 16S rRNA gene sequence analysis places this species in a separate branch of the beta-subclass of *Proteobacteria* most closely related to the genus *Burkholderia* (2). *L. mirabilis* has been recovered from various human sources, but its pathogenic potential is not well understood. Initially, it was isolated from oral and upper respiratory sites (2). Subsequently, it has been isolated as the predominant microorganism from the sputum of a patient with cystic fibrosis (1) and from the oral cavities of children infected with human immunodeficiency virus (4). In order to assist laboratory workers in the identification of this organism, we determined the cellular fatty acid (CFA) composition of the type strain and nine phenotypically similar clinical isolates.

Strains included in the study are listed in Table 1. The type strain was obtained from the American Type Culture Collection, and a previously characterized reference strain was kindly provided by L. Sly (1). Eight additional clinical isolates received by the Centers for Disease Control (CDC) Special Bacteriology Reference Laboratory between 1987 and 2000 were also included. These isolates were identified by traditional bacteriologic methods (5). Cells for fatty acid analysis were grown for 24 h at 35°C on plates of heart infusion agar supplemented with 5% rabbit blood. The cells were harvested, saponified, and processed for total CFAs as described previously (5). The resulting samples of fatty acid methyl esters were analyzed by capillary gas-liquid chromatography. CFA profiles were identified by using a commercially available system (MIDI, New-

ark, Del.). The identification of all acids and the location of the double-bond position of monounsaturated acids were accomplished by combined gas-liquid chromatography-mass spectrometry (5).

The CFA composition of the 10 *L. mirabilis* strains is shown in Table 2. The profiles of all 10 strains tested were qualitatively identical. This profile is characterized by major amounts of 16:1 $\omega$ 7c (34 to 48%) and 16:0 (35 to 48%); smaller amounts (1 to 4%) of 3-OH-10:0, 12:0, 14:0, 15:0, and 18:1 $\omega$ 7c; trace amounts (<1%) of 10:0, 18:2, and 18:0; and no cyclopropane acids. The overall CFA profile of *L. mirabilis* is most similar to those of *Acidovorax delafieldii*, *Comamonas terrigena*, and CDC weak oxidizer group 1 (WO-1) (5).

WO-1 is a group of isolates from a variety of clinical sources first described by Hollis et al. in 1992 (3). The most frequent source of isolation of 96 WO-1 strains studied at CDC is blood (17 of 96 strains) (3). These isolates are phenotypically similar to *A. delafieldii* but can be differentiated by negative reactions for xylose oxidation and citrate alkalization. Molecular classification of this group is pending the receipt of additional isolates. For comparison purposes, the CFA profile of the genus *Acinetobacter* is also shown in Table 2 (5). This organism shares many morphologic and phenotypic characteristics with *L. mirabilis* and has been isolated from the same clinical sources. The high relative proportion (36%) of 18:1 $\omega$ 9c and

TABLE 1. *L. mirabilis* strains used for CFA composition analysis

Strain	Yr received	Location	Source
ACM 3763	1997	Brisbane, Australia	Sputum
ATCC 51599 <sup>T</sup>	2001	Denmark	Gingival margin
F9652	1987	Georgia	Blood
G6766	1991	Georgia	Sputum
G7599	1992	Texas	Sputum
G8736	1993	Missouri	Sputum
G9054	1994	Alabama	Blood
H290	1997	Tennessee	Blood
H1157	1999	New York	Peritoneal fluid
H1619	2000	California	Blood

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TABLE 2. CFA composition of *L. mirabilis*, *C. terrigena*, *A. delafieldii*, CDC group WO-1, and *Acinetobacter* species<sup>a</sup>

Fatty acid	% Total fatty acids <sup>b</sup>					
	<i>L. mirabilis</i> (n = 10)		<i>C. terrigena</i> <sup>c</sup> (n = 1)	<i>A. delafieldii</i> <sup>c</sup> (n = 6)	WO-1 <sup>c</sup> (n = 37)	<i>Acinetobacter</i> species <sup>c</sup> (n = 34)
	Range	Mean				
3-OH-8:0	-	-	-	-	1	-
10:0	T	T	-	-	-	T
3-OH-10:0	1-3	2	5	4	4	-
12:0	2-6	3	4	3	7	7
12:1 $\omega$ 9c	-	-	-	-	-	1
2-OH-12:0	-	-	-	-	-	2
3-OH-12:0	-	-	-	-	-	4
14:0	2-4	3	1	4	2	1
15:1 $\omega$ 6c	-	-	-	1	1	-
15:0	T-3	1	1	1	1	1
3-OH-14:0	-	-	-	-	-	1
16:1 $\omega$ 9c	-	-	-	-	-	1
16:1 $\omega$ 7c	34-48	41	40	38	42	16
16:0	35-48	44	27	27	27	20
1-3-OH-15:0	-	-	-	-	2	-
17:1 $\omega$ 8c	-	-	-	-	-	4
17:0	-	-	1	1	1	2
2-OH-16:0	-	-	2	-	-	-
18:2	T	T	-	2	-	2
18:1 $\omega$ 9c	-	-	-	1	-	36
18:1 $\omega$ 7c	2-6	4	21	16	11	2
18:0	T	T	-	1	T	1

<sup>a</sup> The number before the colon indicates the number of carbons; the number after the colon is the number of double bonds;  $\omega$ , position of the double bond counting from the hydrocarbon end of the carbon chain; OH, a hydroxy group at the 2( $\alpha$ )- or 3( $\beta$ )- position from the carboxyl end; c, *cis* isomer; i, iso; T, 0.4 to 0.8%; -, not detected; n, number of strains tested.

<sup>b</sup> Values are percentages of total fatty acids and are arithmetic means.

<sup>c</sup> Values were obtained from our previous publication (5).

the presence of 2 to 4% amounts of 2-OH-12:0 and 3-OH-12:0 easily differentiates *Acinetobacter* spp. from these other taxa.

The *L. mirabilis* CFA profile contains slightly higher relative amounts of 16:0 (44% versus 20 to 27%) and small (trace to 2%) quantitative differences from the other similar profiles given in Table 2. However, the small number of *C. terrigena* and *A. delafieldii* strains for which profiles were available limits the ability of CFA analysis to provide a definitive identification in the absence of other phenotypic or genetic tests. Therefore, we recommend the use of CFA analysis to place potential *L. mirabilis* isolates in a CFA group that also includes *A. delafieldii*, WO-1, and *C. terrigena*. Tests useful in differentiating *L. mirabilis* from organisms with a similar CFA profile are pre-

sented in Table 3. Coccoid morphology and the ability to ferment D-glucose separates *L. mirabilis* from *C. terrigena*, *A. delafieldii*, and WO-1. Of the three nonfermenters, only *A. delafieldii* is positive for citrate alkalization and for oxidation of D-glucose and D-xylose. The type strain of *C. terrigena* is negative for all three of these tests, whereas WO-1 is positive for D-glucose oxidation but negative for D-xylose oxidation and positive in only 21% of strains for citrate alkalization.

The *L. mirabilis* strains included in this study, like all previously described strains of this species, are of human origin. All previously described strains of this species were obtained from mixed-flora sites (oral cavity, sputum, upper respiratory tract) where the clinical significance of the organism is not easily

TABLE 3. Biochemical differentiation of *L. mirabilis*, *C. terrigena*, *A. delafieldii*, and CDC group WO-1<sup>a</sup>

Test	Differentiation of:			
	<i>L. mirabilis</i> (n = 10)	<i>C. terrigena</i> (type strain)	<i>A. delafieldii</i> (n = 6)	CDC group WO-1 (n = 37)
Coccoid morphology	+	-	-	-
Fermentation of D-glucose (acid in TSI butt)	+	-	-	-
Acid from (OF media):				
D-Glucose	+	-	+	+
D-Xylose	-	-	+	-
Citrate alkalization	-	-	+	v

<sup>a</sup> Results are based on methods described in reference 5. TSI, triple sugar iron agar; OF, King's oxidation-fermentation medium; +, 90% or more strains tested positive; -, 10% or fewer strains tested positive; v, 11 to 89% strains tested positive; n, number of strains.

determined (1, 2, 3). Gerner-Smidt et al. suggested a possible role for this organism in the development of dental plaque (2). Our results suggest a potential for *L. mirabilis* to cause invasive disease, since five of our clinical isolates were obtained from normally sterile sites (blood, peritoneal fluid). Detailed case reviews, in conjunction with virulence and host susceptibility studies, will be required to better define the pathogenic spectrum of this species.

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