Cellular Fatty Acids of Brucella canis and Brucella suis

SALLY B. DEES,* DANNIE G. HOLLIS, ROBERT E. WEAVER, AND C. WAYNE MOSS

Bacteriology Division, Centers for Disease Control, Atlanta, Georgia 30333

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The cellular fatty acid composition of Brucella canis and Brucella suis was determined by gas-liquid chromatography. The presence of relatively large amounts of a 19-carbon cyclopropane fatty acid in B. suis was a major distinguishing feature between these organisms. The gas-liquid chromatography test for cellular fatty acids provides an additional criterion for the distinction of antigenically rough strains of B. suis which cannot be differentiated from B. canis by conventional procedures.

Four species of the genus Brucella, B. abortus, B. melitensis, B. suis, and B. canis, have been associated with disease in humans (3). Differentiation of these species is generally straightforward through use of conventional microbiological and serological procedures (1). Difficulty arises when antigenically rough strains of B. suis, B. abortus, and B. melitensis are encountered in clinical specimens. Most isolates of these species are antigenically smooth and are readily distinguished from B. canis which exists only in the rough form. In contrast, rough strains of these organisms resemble B. canis in that they agglutinate in antirough Brucella species serum and acriflavine and are not susceptible to phage lysis. Whereas the slow hydrolysis of urea (1 to 2 h) by B. abortus and the growth of B. melitensis in dilute basic fuchs in (1:50,000) distinguish these two species from B. canis, none of the conventional tests are adequate for the differentiation of B. suis (1). Thus, additional criteria are needed to distinguish rough strains of B. suis from those of B. canis. In previous reports, we have shown that certain closely related bacteria can be differentiated on the basis of cellular fatty acid composition (6). In this report, we compare the cellular fatty acids of representative strains of B. canis and B. suis.

Seven well-characterized strains of B. canis which were isolated from dogs or humans and included strain RM666 from L. E. Carmichael and five human isolates of B. suis were studied. In addition, two human isolates of Brucella species which were antigenically rough and had been tentatively identified by conventional biochemical tests as B. suis were tested. Cells for fatty acid analysis were grown on plates of heart infusion agar enriched with 5% rabbit blood and incubated in a candle jar for 24 or 48 h at 35°C. Cells were scraped from the plates by using approximately 0.5 ml of sterile distilled water; they were then placed in test tubes (18 by 150 mm) with Teflon-lined caps and processed for cellular fatty acids by previously published procedures (4). The fatty acid methyl esters were analyzed by gas-liquid chromatography on a 3% SE-30 column with a flame ionization detector (7). Bacterial fatty acids were tentatively identified by comparison of gas-liquid chromatography retention times to authentic standards (Applied Science, State College, Pa.; Analabs, North Haven, Conn.), and their identities were confirmed by mass spectrometry (8). Procedures for acetylation, hydrogenation, and calculation of relative peak areas of the fatty acid methyl esters have been described earlier (4, 7).

The fatty acid profiles of representative strains of the two species are shown in Fig. 1. The presence of relatively large amounts of a 19-carbon cyclopropane acid (19:0 CYC) in B. suis (bottom chromatogram) was the major distinguishing feature. This acid was present in concentrations of 35 to 50% of the total cellular acids in B. suis but was not detected in B. canis in more than trace amounts (Table 1). The major acid in B. canis (top chromatogram) was 18:1 which was present at concentrations of approximately 60% (Table 1). The other major acids in this organism were 16:0 (24%), 18:0 (16.1%), 18:2 (10%), and 18:1 (7%). Moderate amounts of 16:0 (18%) and 18:0 (14%) were also detected in B. suis. Small to trace amounts of 17:0 and 17:1 CYC acids were present in both species; only trace amounts of 16:1 in B. suis. No branch-chain or hydroxy-substituted fatty acid was detected in either species. The antigenically rough strains tentatively identified as B. suis were found to be essentially identical to smooth strains of B. suis in that they contained 19:0 CYC as the major distinguishing acid, whereas
 TABLE 1. Cellular fatty acid composition of B. canis and B. suis

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<th>Organism</th>
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<td>16:1°C 16:0 17:0 CYC 17:0 18:1 18:0 19:0 CYC 19:0</td>
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<tr>
<td>B. canis (7)*</td>
<td>24 h 7 24 T T 59 10 T —</td>
</tr>
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<td>48 h 8 24 T T 58 10 T —</td>
</tr>
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<td>B. suis (7)</td>
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*— Less than 2%.
° Number to left of colon refers to number of carbon atoms; number to right refers to number of double bonds.
°° Number in parentheses refers to number of strains tested.

This acid was not detected in B. canis. The fatty acid data clearly support the identification of these strains as B. suis. Although additional rough strains need to be tested, our data indicate that the gas-liquid chromatography test for cellular fatty acids is a major differentiating criterion for the distinction of rough strains of B. suis from B. canis.

Since the relative amounts of cyclopropane acids in bacteria are known to increase with culture age (2), strains of each species were processed for cellular fatty acids after 24 and 48 h of growth. Data in Table 1 show that the relative concentration of 19:0 CYC in B. suis increased from 35% in 24-h cells to 50% in 48-h cells. This increase was accompanied by a concomitant decrease in 18:1 (31% to 16%), indicating that this acid serves as the direct precursor of the 19:0 CYC acid. The 19:0 CYC acid was completely absent or present in only trace amounts in cultures of B. canis, regardless of the length of incubation. Thus, the absence of 19:0 CYC acid in B. canis is useful for rapid identification of this organism since all other Brucella species contain relatively large amounts of this acid (5, 9).

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