Differentiation of *Bordetella avium* and Related Species by Cellular Fatty Acid Analysis

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The fatty acids of 18 strains of *Bordetella avium*, 3 strains of *Alcaligenes faecalis*, 5 strains of *Bordetella bronchiseptica*, and 12 strains of a *B. avium*-like organism were examined by gas chromatography-mass spectrometry. The presence of a significant amount of the acid 2-OH C14:0 characterized *B. avium* and the *B. avium*-like organism. *B. avium* and the *B. avium*-like organism differed in their relative concentrations of C16:1 and 3-OH C14:0 acids. *B. bronchiseptica* and *A. faecalis* were distinguishable by comparison of the relative concentrations of C18:0 and C18:1 acids.

In the late 1970s a gram-negative, motile, asaccharolytic bacterium was associated with an acute respiratory disease of turkey poults (5). The disease has since been reported by other workers, although different terms were used, e.g., rhinotracheitis (14) and bordetellosis (6).

Considerable confusion and disagreement have occurred concerning the classification of the disease agent. It has been termed *Bordetella*-like (6), *Alcaligenes faecalis* (15), and *Bordetella meleagris* or *Alcaligenes meleagris* (13). Kersters et al. (11) demonstrated that the agent is a member of the genus *Bordetella* and proposed the new species *Bordetella avium*. In addition, recent studies have indicated that an organism very similar to *B. avium* also occurs in chickens and turkeys (1, 7, 13; P. J. Blackall and C. M. Doheny, Aust. Vet. J., in press). We have adopted the suggestion of Jackwood et al. (8) and use the term *B. avium*-like for this organism.

A major reason for the confusion over the identification and classification of *B. avium* is that this organism is relatively inert and hence the conventional tests yield mainly negative results. These negative results make separation of *B. avium* from other similar inert organisms such as *A. faecalis* or *Bordetella bronchiseptica* difficult.

In recent years, gas chromatographic profiling of the cellular fatty acid composition of bacterial cells has been successfully used to classify and identify many bacteria (9). In particular, the technique has been used to identify members of the genera *Alcaligenes* (4) and *Bordetella* (3, 10). Recently, Jackwood et al. (8) described, for the first time, the fatty acid profiles of *B. avium* and the *B. avium*-like organism.

This report presents the results of our gas chromatography-mass spectrometry studies of the cellular fatty acid composition of *B. avium*, the *B. avium*-like organism, *A. faecalis*, and *B. bronchiseptica*. They provide some criteria for the separation of these four organisms based on their fatty acid profiles. These criteria are particularly useful for laboratories with access to high-resolution (capillary) gas chromatographs but lacking the software necessary for rapid probability-based identification. In addition, we clarify some ambiguities in the literature concerning the fatty acid profiles of members of the genus *Bordetella*.

**MATERIALS AND METHODS**

**Cultures.** The field isolates of *B. avium* (12 strains) and the *B. avium*-like organism (10 strains) have been described elsewhere (2; Blackall and Doheny, in press). The reference strains used are given in Table 1.

**Cell preparation and derivative formation.** Bacteria were grown on brain heart infusion agar (GIBCO Laboratories, Brisbane, Australia) with 5% sheep blood for 16 h at 37°C in an atmosphere of 5% CO2. The confluent growth from three plates was gently harvested in sterile distilled water and washed once, and the cells were resuspended in 3 ml of sterile distilled water divided into 1-ml aliquots, and stored at −20°C. The fatty acid methyl esters were then prepared (12).

**Gas chromatography-mass spectrometry.** Gas chromatographic-mass spectrometric analysis was performed on a Finnigan 1020B spectrometer equipped with a data system and a National Bureau of Standards library. Chromatography was performed on a DB-5 fused silica column (30 m by 0.25 mm) (J & W Scientific Inc., Rancho Cordova, Calif.) interfaced directly to the mass spectrometer ion source. Injector and interface ovens were maintained at 250°C. Injection was splitless (2 s), with an oven temperature of 120°C. After 2 min, the oven temperature was raised by a linear gradient of 7°C/min to 260°C, where it was maintained for 8 min. The total analysis time was 30 min. The carrier gas was helium, with a linear flow rate of 30 cm/s at 120°C. The mass spectrometer was operated in the electron impact mode and scanned from 35 to 350 atomic mass units in 1 s. Identification of peaks was performed by using the library search facility, by comparison with published spectra, or by analysis of authentic material. Quantification was performed by the software by using a retention time window of 800 s, sufficient to include all fatty acid methyl esters from C10:0 to C19:0. This resulted in over 50 components commonly being included in the quantification with the major components (Table 2), constituting ca. 85% of the total integrated area.

**RESULTS**

The range of relative concentrations of cellular fatty acids detected in the *B. avium*, *B. avium*-like organism, *A. faecalis* and *B. bronchiseptica* strains are presented in Table 2. We found little difference for the isolates within each of the four groups. The chromatograms of the type strains of the three
TABLE 1. Identification, origins, and sources of reference strains

<table>
<thead>
<tr>
<th>Organism</th>
<th>Origin</th>
<th>Source*</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. avium</td>
<td>270-80 Goose, Federal Republic of Germany</td>
<td>Hinz</td>
</tr>
<tr>
<td></td>
<td>383-78 Turkey, Federal Republic of Germany</td>
<td>Hinz</td>
</tr>
<tr>
<td></td>
<td>591-77 Turkey, Federal Republic of Germany</td>
<td>Hinz</td>
</tr>
<tr>
<td>P-8</td>
<td>Turkey, United States</td>
<td>Simmons</td>
</tr>
<tr>
<td>002</td>
<td>Turkey, United States</td>
<td>Jackwood</td>
</tr>
<tr>
<td>197</td>
<td>Turkey, United States</td>
<td>Jackwood</td>
</tr>
<tr>
<td>B. avium-like</td>
<td>128 Turkey, United States</td>
<td>Jackwood</td>
</tr>
<tr>
<td></td>
<td>154 Turkey, United States</td>
<td>Jackwood</td>
</tr>
<tr>
<td>B. bronchiseptica</td>
<td>186 Turkey, United States</td>
<td>Jackwood</td>
</tr>
<tr>
<td></td>
<td>188 Turkey, United States</td>
<td>Jackwood</td>
</tr>
<tr>
<td></td>
<td>ATCC 19395 Dog</td>
<td>ATCC</td>
</tr>
<tr>
<td></td>
<td>ATCC 4617 NK</td>
<td>ATCC</td>
</tr>
<tr>
<td></td>
<td>NCTC 8344 NK</td>
<td>NCTC</td>
</tr>
<tr>
<td>A. faecalis</td>
<td>ATCC 8750 NK</td>
<td>ATCC</td>
</tr>
<tr>
<td></td>
<td>NCIB 9650 NK</td>
<td>UQM</td>
</tr>
<tr>
<td></td>
<td>ATCC 19018 NK</td>
<td>Glfenfeld</td>
</tr>
</tbody>
</table>

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recognized species are shown in Fig. 1. The peak marked X is an artifact most probably derived from the C17 cyclopropane (cyc) acid during esterification.

The cellular fatty acids of the three species and the B. avium-like organisms were qualitatively similar. The major fatty acids present were C12:0, C14:0, C15:0, C16:0, C16:1, C17:0, C17 cyc, C18:0, C18:1, and the hydroxy acids 2-OH C12:0 and 3-OH C14:0. The acids C16:0 and C17 cyc constituted about 60% of the total cellular fatty acids for all of the isolates examined.

Examination of the cellular fatty acid profiles allowed the four groups of organisms to be clearly separated. All the B. avium and B. avium-like isolates had 2-OH C14:0 content which, although small, was much larger than that of any A. faecalis or B. bronchiseptica isolate. The B. avium-like isolates could be separated from B. avium by a comparison of the ratios of C16:1 and 3-OH C14:0 acids. All B. avium isolates had a C14:0 content lower than that of the closely adjacent 3-OH C14:0, whereas all the B. avium-like isolates had a C16:1 content higher than the 3-OH C14:0 content.

B. bronchiseptica and A. faecalis were most easily separated by comparison of the ratios of C18:0 and C18:1 acids. The three A. faecalis isolates showed a C18:1 content greater than the C18:0 content. For the five B. bronchiseptica isolates, the reverse was true, i.e., the C18:0 content was higher than the C18:1 content. All the isolates of B. avium and the B. avium-like organisms also had a C18:0 content greater than the C18:1 content.

The means of the concentrations of the fatty acids useful in separating the four taxa (3-OH C14:0, C16:1, C18:1, and C18:0) are presented in Table 3.

DISCUSSION

The cellular fatty acid contents, as determined by gas chromatography-mass spectrometry, allowed the ready recognition of the four taxa examined in this study, B. avium, the B. avium-like organism, A. faecalis, and B. bronchiseptica. Although the four taxa all had C16:0 and C17 cyc as the major fatty acids, the ratios of the minor components clearly separated the organisms (Table 2 and Fig. 1).

Our findings are, in general, in agreement with those of a similar study performed by Jackwood et al. (8). In both studies, B. avium and the B. avium-like organism were readily distinguished from A. faecalis and B. bronchiseptica by the presence of significant amounts of 2-OH C14:0. However, Jackwood et al. (8) based their differentiation of B. avium and the B. avium-like organism on statistically significant differences between their C16:0/C14:0 ratios and by use of the Hewlett-Packard 5898A microbial identification system. In contrast, our results suggest a much more convenient distinguishing feature: B. avium isolates had a C16:1 content lower than that of 3-OH C14:0, whereas B. avium-like isolates had a C16:1 content higher than that of 3-OH C14:0.

Our examination of 18 B. avium isolates and 12 B. avium-like isolates from three continents showed the C16:1/3-OH C14:0 ratio to be a simple and completely reliable method of separating B. avium and the B. avium-like organism. We emphasize that this subtle but significant difference between B. avium and the B. avium-like organism necessitates strict adherence to our conditions of growth and chemical workup. Further work on the effects of various incubation conditions and different media is planned.

In agreement with Jackwood et al. (8), we found that the avian isolates of B. bronchiseptica possessed fatty acid profiles very similar to those of the reference strains obtained from other animals.

There has been some disagreement concerning the C18:0 and C18:1 content of members of the genus Bordetella. In the study by Jantzen et al. (10) of 13 Bordetella pertussis isolates, 3 Bordetella parapertussis isolates, and 7 B. bronchiseptica isolates, the chromatograms illustrated show a C18:0 content greater than the C18:1 content. However, in

TABLE 2. Cellular fatty acid composition of strains of B. avium, the B. avium-like organism, B. bronchiseptica, and A. faecalis

<table>
<thead>
<tr>
<th>Organism (no. of strains)</th>
<th>C12:0</th>
<th>2-OH C12:0</th>
<th>C14:0</th>
<th>2-OH C14:0</th>
<th>C15:0</th>
<th>C16:0</th>
<th>C16:1</th>
<th>C17:0</th>
<th>C17 cyc</th>
<th>C18:0</th>
<th>C18:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. avium (18)</td>
<td>0.4-1.0</td>
<td>1.4-2.1</td>
<td>0.6-0.9</td>
<td>1.5-2.6</td>
<td>3.0-4.6</td>
<td>0.1-0.4</td>
<td>28.0-36.0</td>
<td>0.8-2.8</td>
<td>0.8-2.1</td>
<td>19.0-30.0</td>
<td>7.0-13.0</td>
</tr>
<tr>
<td>B. bronchiseptica (5)</td>
<td>0.5-1.0</td>
<td>1.5-3.7</td>
<td>4.3-7.2</td>
<td>&lt;0.2</td>
<td>3.5-5.3</td>
<td>0.4-1.4</td>
<td>35.0-42.0</td>
<td>6.0-17.0</td>
<td>0.9-2.0</td>
<td>14.0-22.0</td>
<td>3.0-7.0</td>
</tr>
<tr>
<td>A. faecalis (3)</td>
<td>1.0-2.0</td>
<td>1.0-2.5</td>
<td>0.5-1.5</td>
<td>&lt;0.2</td>
<td>4.3-8.2</td>
<td>0.2-1.2</td>
<td>34.0-40.0</td>
<td>6.5-14.0</td>
<td>0.8-1.0</td>
<td>15.0-23.0</td>
<td>1.5-2.7</td>
</tr>
<tr>
<td>B. avium-like (12)</td>
<td>0.4-1.1</td>
<td>1.0-3.3</td>
<td>0.6-1.3</td>
<td>1.5-4.0</td>
<td>3.4-5.1</td>
<td>0.2-0.4</td>
<td>31.0-38.0</td>
<td>5.5-12.0</td>
<td>0.9-2.4</td>
<td>11.0-23.0</td>
<td>5.0-9.0</td>
</tr>
</tbody>
</table>
the table accompanying the chromatograms, the values for B. bronchiseptica and B. parapertussis indicate a reverse ratio, i.e., C18:1 content greater than C18:0 content. Dees et al. (3) reported similar levels of C18:0 and C18:1 in five isolates of B. bronchiseptica. Jackwood et al. (8) reported that 13 B. bronchiseptica isolates and 5 B. parapertussis isolates were all characterized by a C18:1 content greater than the C18:0 content. However, for 59 B. avium isolates and 46 B. avium-like isolates, Jackwood et al. (8) reported the opposite ratio, i.e., C18:0 content greater than C18:1 content. In contrast, we found that all Bordetella isolates we examined (18 B. avium isolates, 5 B. bronchiseptica isolates, and 12 B. avium-like isolates) had a C18:0 content greater than the C18:1 content.

It is possible that peak X in Fig. 1, clearly identifiable as an artifact by mass spectrometry and most probably derived from C17 cyc during esterification, may have contributed to this confusion concerning the C18:0/C18:1 ratio. The identities and origins of this and similar artifacts have been described by Vulliet et al. (16). The major fragments in its mass spectrum are at m/e 129 and m/e 201. Laboratories basing fatty acid identification on retention time data could misidentify this artifact as a C18:1 isomer. Our results show that a C18:1 isomer was occasionally detectable superimposed on the artifact. In such instances, it was present only in trace quantities (<0.2%).

It is likely that for bacterial species in which the C17 cyc component is at a low level or absent, no ambiguity due to the artifact would arise. This may explain why Jantzen et al. (10) reported that the C18:0 content of B. pertussis, an organism containing only trace levels of C17 cyc, was greater than the C18:1 content.

In species in which the C18:0 component is sufficiently large, the mistaken inclusion of the artifact as a C18:1 isomer would not result in a C18:1 content greater than the C18:0 content. This may explain why Jackwood et al. (8) reported that all their B. avium and B. avium-like isolates had a C18:0 content greater than the C18:1 content yet reported the opposite for their B. bronchiseptica and B. parapertussis isolates.

In contrast to our results for the three Bordetella taxa, we found that the three A. faecalis isolates had the reverse ratio of C18:0 and C18:1 acids, i.e., a C18:1 content greater than the C18:0 content. Previous studies of 29 A. faecalis isolates, 4 Alcaligenes denitrificans isolates, and 11 "Alcaligenes odorans" isolates have all reported a similar finding (3, 4, 8).

Our results suggest that when isolates of A. faecalis, B. bronchiseptica, B. avium, and the B. avium-like organism are examined for cellular fatty acids by using the growth medium and conditions we describe, the ratios of C18:0 and C18:1 fatty acid contents provide a clear distinction between the two genera.

As also reported by Jackwood et al. (8), we found that all of our isolates of B. avium and the B. avium-like organism possessed significant amounts of 2-OH C14:0. In contrast, our
A. faecalis and B. bronchiseptica isolates possessed only trace amounts of this acid. Other studies have confirmed the absence of this acid in other members of the genus Bordetella (3, 8, 10).

Small amounts of 2-OH C14:0 have been found in some isolates of A. faecalis (4, 8). This finding would still not confuse the identification of B. avium or the B. avium-like organism. The ratio of the C16:0 and C18:1 content would still clearly separate any 2-OH C14:0-containing A. faecalis isolates from B. avium or the B. avium-like organism.

In summary, we confirmed that the four taxa, B. avium, the B. avium-like organism, B. bronchiseptica, and A. faecalis possess distinct cellular fatty acid profiles. The three members of the genus Bordetella could be distinguished from A. faecalis by comparison of the relative amounts of C18:0 and C18:1 acids present. B. avium and the B. avium-like organism contained the acid 2-OH C14:0 at much higher levels than did B. bronchiseptica or A. faecalis. B. avium and the B. avium-like organism could be separated by a comparison of the relative amounts of C16:1 and 3-OH C14:0 acids. For diagnostic veterinary laboratories with access to a high-resolution gas chromatograph, the detection of cellular fatty acid profiles offers a rapid and reliable method for the identification of B. avium and the B. avium-like organism.

**ACKNOWLEDGMENTS**

This work was supported in part by a grant from the Australian Chicken Meat Research Committee.

The technical assistance of C. Doheny and D. Rogers is gratefully acknowledged. We acknowledge the assistance of E. Jantzen, who drew our attention to the report of Vulliet et al. (16) on the occurrence of cyclopropane fatty acid-derived artifacts.

**LITERATURE CITED**


