

Differentiation of *Brucella canis* from Other Brucellae by Gas Chromatography

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Gas chromatographic techniques allow for differentiation between a strain of *Brucella canis* and strains of other brucellae.

A gram-negative coccobacillary bacterium isolated from placental and fetal tissues of an aborted beagle pup was characterized as a new species of *Brucella*, and the name *Brucella canis* was proposed for this previously unrecognized bacterium (1). The present study was designed to determine whether gas chromatographic techniques may be employed to distinguish between a strain of *B. canis* and isolates of other *Brucella* species.

B. abortus 544, *B. melitensis* 16 M, *B. ovis* REO-1182, *B. suis* 1330, dog abortion agent RM 6 66 (designated herein as *B. canis*) obtained from the amniotic fluid of an aborted beagle fetus, and *Bordetella bronchiseptica* were grown at 37 C on slants containing 1.5% tryptone, 1.5% yeast extract, 0.5% glucose, 0.5% K_2PHO_4 , and 1.5% agar (TYG medium). After 24 hr, the cells were washed from the agar with 5.0 ml of distilled water, the bacterial suspension was diluted, and 0.10 ml containing 25×10^3 to 250×10^3 cells was transferred into a screw-capped test tube containing 5.0 ml of TYG broth. After 24 hr of incubation at 37 C, replicates were examined microscopically for counts, and the cultures and samples of uninoculated medium were treated with 0.10 ml of 5 N HCl and 1.0 ml of 0.2 M HCl-KCl buffer (pH 2.0). The samples were then centrifuged at $3,000 \times g$, and the supernatant fluid was extracted three times with 10 ml of ether. The extracts were combined, concentrated to 0.5 ml, and dried with anhydrous Na_2SO_4 . A 3.0- μ liter sample was injected into the gas chromatograph. The chromatographic techniques were essentially the same as described previously (2).

The differentiating peaks of these strains of *Brucella* and *Bordetella* are shown in Table 1. With the electron capture detector to record the presence of microbial metabolites, the sensitivity of detection of the bacteria ranged from 42 to 490 organisms per 10 mm² peak area. The sensi-

tivity was calculated from the number of cells in the 24-hr sample which was injected into the chromatograph and the peak area of the product, the value given for sensitivity being the estimated number of bacteria to yield a peak area of 10 mm². Each *Brucella* strain exhibited 10 to 15 peaks in chromatograms prepared from the spent culture media. Peaks having retention times of 25, 35, 45, 55, 60, 75, 85, 90, 390, and 940 sec were common to most of the organisms. However, as shown in Table 1, at least one compound

TABLE 1. Detection of differentiating peaks of *Brucella* species by electron capture detector

Bacteria	Cells/ml		Sensitivity ^a	Characteristic peaks ^b
	0 hr	24 hr		
<i>Brucella abortus</i> ...	5.0×10^4	2×10^2	42	960
<i>B. melitensis</i>	5.0×10^4	5×10^2	125	280
<i>B. suis</i>	5.0×10^4	80×10^2	150	65
<i>B. canis</i>	5.0×10^4	90×10^2	124	1,400
<i>B. ovis</i>	5.0×10^4	60×10^2	205	185; 1,540
<i>Bordetella bronchiseptica</i>	50.0×10^3	11×10^2	490	160

^a Bacteria/10 mm² peak area.

^b Retention times in seconds.

was present in cultures of each *Brucella* strain which was not produced by any other organism tested. These metabolites were not found in the uninoculated medium, and the area of these ranged from 500 to 1,710 mm².

A signature for each *Brucella* strain was established by assigning letters to peaks in the chromatogram in order of their increasing retention times. By means of the signatures obtained, the strains examined could be characterized readily. When products elaborated by *B. canis* were compared with those of the other bacteria investigated, the dog pathogen was found to differ from *B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, and *B.*

bronchiseptica by eight, nine, three, eight, and four products, respectively. Some similarity in the kinds of substances excreted was evident, therefore, between *B. canis* and *B. suis* as well as *B. bronchiseptica*. On the other hand, most of the products formed by *B. canis* as well as the other brucellae were quite distinct from those synthesized by strains of *Bacillus*, *Clostridium*, and *Staphylococcus*.

The results thus indicate that *B. canis* excreted metabolites similar to those generated by strains of other *Brucella* species. However, the brucellae investigated, although admittedly few in number, can be readily differentiated from one another by

considering the presence or absence of individual compounds detectable by gas chromatography.

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