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Direct Hemagglutination Technique for Differentiating *Bacteroides asaccharolyticus* Oral Strains from Nonoral Strains

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A simple and economical method for differentiating *Bacteroides asaccharolyticus* of oral sources from nonoral sources is described. The present data indicate that oral strains of *B. asaccharolyticus* strongly agglutinate sheep erythrocytes, whereas isolates from various nonoral sites typically are devoid of hemagglutination activity. The direct hemagglutination test may aid in determining the source of *B. asaccharolyticus* present in an infection, and thus the procedure has potential value as a means of biotyping.

Bacteroides melaninogenicus and *Bacteroides asaccharolyticus* are commonly isolated from the oral cavity, the intestinal tract, and the genitalia. These species play an essential role in the pathogenesis of experimental infections established with mixtures of anaerobic bacteria (5). Strains of the *B. melaninogenicus/B. asaccharolyticus* group, in particular, *B. asaccharolyticus*, have been implicated as significant pathogens in a variety of serious human infections (2). The source of the infectious organisms can be determined with a high degree of certainty in wound infections in which a direct implantation into the tissue has taken place. On the other hand, the source of the pathogens is often unknown when bacteremia occurs and the microorganisms infect usually sterile areas. Tooth extraction, oral surgery, oral hygiene procedures, and even chewing can produce a transient bacteremia of bacteroides and other oral organisms (1). Therefore, the possibility exists that the primary locus for pathogenic components of nonoral infections can be the oral cavity. The present report demonstrates that a direct hemagglutination technique can be useful in determining whether strains of *B. asaccharolyticus* originate from oral or various nonoral sites.

MATERIALS AND METHODS

Bacteria. A total of 70 strains of *B. asaccharolyticus* were used to evaluate hemagglutination activity. They were gram-negative anaerobic rods which produced black-pigmented colonies on Todd-Hewitt agar (BBL Microbiology Systems) supplemented with 10% rabbit blood, 5 μg of hemin per ml, and 0.2 μg of menadione per ml (7). They fermented no carbohydrates and produced indole. Acetic, propionic, isobutyric, butyric, and isovaleric acids were detected in acidified cultures of peptone-yeast extract-glucose broth (4).

Fifty-four of the 70 isolates tested were obtained from samples of supragingival and subgingival plaque of 22 periodontitis patients. These oral strains included fresh isolates and strains obtained from our stock collection. Several strains were subcultured numerous times before the present experiment.

The remaining 16 strains were isolated from nonoral sites in humans. Two fecal strains were recent isolates from our laboratory. The following seven strains of fresh clinical isolates were received through the courtesy of K. J. Wicher, Buffalo, N.Y.: two strains originated from cervix; one strain each originated from umbilicus, amniotic fluid, pelvic abscess, pilonidal cyst, and blood. D. W. Lambe, Johnson City, Tenn., kindly provided strains 1070-70 (source unknown), 783-75A (left flank abscess), 750-75G (ear tissue), and 785-75A (mastoid tissue). One fecal isolate (B 536) was obtained from S. M. Finegold, Los Angeles, Calif. Strains ATCC 25260 (empyema) and ATCC 27067 (human leg wound) were received from the American Type Culture Collection, Rockville, Md. Cultures either were maintained on blood agar or were frozen at −80°C.

Hemagglutination of sheep erythrocytes. Bacteria were harvested from 5 to 7 days of growth on blood agar and from 2 to 3 days of growth in prereduced anaerobically sterilized Todd-Hewitt broth (BBL) supplemented with 5 μg of hemin per ml and 0.2 μg of menadione per ml (7). The bacterial cells from the broth culture were washed twice in 0.15 M NaCl buffered at pH 7.2 with 0.016 M Na_2HPO_4 and 0.003 M KH_2PO_4 (PBS) and suspended in PBS at a concentration of 10^9 cells per ml. Erythrocytes from defibrinated sheep blood were centrifuged at 800 × *g*, washed twice in PBS, and resuspended in PBS at a 3% (vol/vol) concentration.

Hemagglutination activity was determined for each strain by use of slide tests and microtitration plates.

The slide tests were performed using harvested cells from growth on blood agar. Cells sufficient to give a concentration of 10^9 were emulsified in a drop (0.05 ml) of PBS on a glass microscope slide. Likewise, 1 drop of the washed bacterial suspension originating from the broth cultures was placed on a glass slide. One drop of the sheep erythrocyte suspension was added to the bacterial emulsions. The slides were gently rotated until hemagglutination occurred or for several minutes if the erythrocytes failed to agglutinate.

The hemagglutination test in microtitration plates was carried out as follows: a sample (0.05 ml) of the bacterial suspension was mixed with 0.05 ml of the washed erythrocyte suspension. After 30 min of shaking at room temperature, the mixture was stored overnight at 4°C.

Agglutination was routinely determined with a dissecting microscope at ×30 magnification and in wet-mount preparations at ×125 magnification. Strains which exhibited no hemagglutination activity were recultured for testing at least four to five times before they were designated hemagglutination negative.

RESULTS

All 54 strains of *B. asaccharolyticus* recovered from the oral cavity exhibited a distinct hemagglutination activity when mixed with sheep erythrocytes. The hemagglutination took place with both the slide tests and the microtitration plate method. In general, the hemagglutination was strongest, often occurring within 30 to 60 s of mixing, when the bacterial cells originated from growth on blood agar and when a slide test was carried out (Fig. 1a).

By contrast, the 16 strains of *B. asaccharolyticus* of nonoral origin failed to agglutinate sheep erythrocytes (Fig. 1b). The absence of hemagglutinating activity of these nonoral strains was a consistent finding observed for each culture examined in each of the various assays for testing hemagglutination activity.

DISCUSSION

The results of this study confirm and extend previous results of Slots and Gibbons (7). These authors found that human erythrocytes were consistently agglutinated by oral strains of *B. asaccharolyticus*, whereas oral *B. melaninogenicus* strains failed to agglutinate erythrocytes. An implication of their findings was that the hemagglutination activity test may be a rapid and reliable method for distinguishing *B. asaccharolyticus* from *B. melaninogenicus* derived from the oral cavity. Their study only included one nonoral strain of *B. asaccharolyticus*, and no conclusion could therefore be made on the relationship of the hemagglutination activity between oral and nonoral strains of *B. asaccharolyticus*.

The present investigation examined 16 strains of *B. asaccharolyticus* isolated from healthy and diseased nonoral sites. A significant finding was that none of these *B. asaccharolyticus* strains from nonoral sources agglutinated sheep erythrocytes. Nonoral strains of *B. melaninogenicus* belonging to *B. melaninogenicus* subsp. *intermedius* (five strains) and *B. melaninogenicus* subsp. *melaninogenicus* (two strains) were also found not to agglutinate the sheep erythrocytes. In contrast, the *B. asaccharolyticus* strains of oral origin all exhibited a strong hemagglutination activity. Hemagglutination occurred with fresh isolates and with stock cultures which had been subcultured numerous times. Therefore, hemagglutination activity is probably a stable property of oral *B. asaccharolyticus*.

The hemagglutination activity of *B. asaccharolyticus* has been attributed to pili (6). Nonhemagglutinating strains of oral *B. melaninogenicus* also possess pili (7), but the surface components of these strains are obviously of a different nature than those of oral *B. asaccharolyticus*. The biochemical characteristics of *B. asaccharolyticus* and *B. melaninogenicus* vary considerably (3); therefore, different cell surface compositions could be expected. Perhaps more unpredictable was the present finding of differences in surface properties of *B. asaccharolyticus* from oral and nonoral sites. It should be noted that marked differences in the cell surface of various "ecotypes" of *B. asaccharolyticus* have been shown in recent immunological studies using an indirect fluorescent-antibody technique (C. Mouton, J. Slots, and R. J. Genco, J. Dent. Res., Special Issue A, Int. Assoc. Dent. Res. no. 56, 1979) and gel precipitation assays (M. J. Reed, J. Slots, C. Tylenda, and R. J. Genco, J. Dent. Res., Special Issue A, Int. Assoc. Dent. Res. Abstr. no. 57, 1979). Further studies are needed to elucidate the possible ecological and pathological significance of the cell surface differences of various ecotypes of *B. asaccharolyticus*. Also, a study on the deoxyribonucleic acid relatedness of hemagglutination-positive versus hemagglutination-negative strains of *B. asaccharolyticus* appears warranted.

Different surface properties of oral and nonoral *B. asaccharolyticus* strains may aid in determining the primary source of this microorganism in a clinical setting. The present data, therefore, may have practical value.

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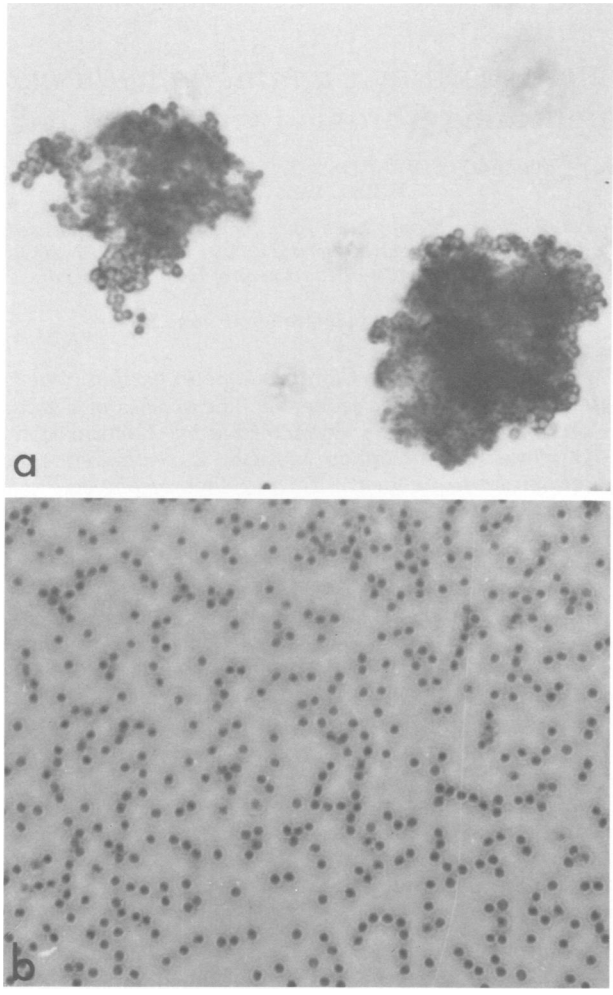

FIG. 1. *(a) Slide hemagglutination with sheep erythrocytes and an oral strain of B. asaccharolyticus grown on blood agar (×125). (b) Lack of agglutination between sheep erythrocytes and a nonoral strain of B. asaccharolyticus grown on blood agar (×125).*

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