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 esti 
{mated with mixtures of anaerobic bacteria (5). Strains of the B. melaninogenicus/B. asaccha 
rolyticus 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 cer 
tainty 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 pro 
cedures, and even chewing can produce a tran 
sient bacteremia of bacteroides and other oral 
organisms (1). Therefore, the possibility exists 
that the primary locus for pathogenetic com 
ponents of nonoral infections can be the oral cavity. 
The present report demonstrates that a direct 
hemagglutination technique can be useful in de 
termining 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 pro 
duced black-pigmented colonies on Todd-Hewitt agar 
(BBL Microbiology Systems) supplemented with 1% 
rabbit blood, 5 μg of hemin per ml and 0.2 μg of 
menadione per ml (7). They fermented no carbohy 
drates and produced indole. Acetic, propionic, isobu 
tyric, 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 cour 
tesy of K. J. Wicher, Buffalo, N.Y.: two strains origi 
nated 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 Cul 
ture Collection, Rockville, Md. Cultures either were 
maintained on blood agar or were frozen at −80°C.

Hemagglutination of sheep erythrocytes. Bac 
teria were harvested from 5 to 7 days of growth on 
blood agar and from 2 to 3 days of growth in prere 
duced 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 Na2HPO4, and 
0.003 M KH2PO4 (PBS) and suspended in PBS at a 
concentration of 10⁹ 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 10⁶ 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 wetmount 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). Non-hemagglutinating 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 pathologic 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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ORAL AND NONORAL B. ASACCHAROLYTICUS STRAINS

LITERATURE CITED