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Long-Wave UV Light Fluorescence for Identification of Black-Pigmented *Bacteroides* spp.

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Black-pigmented *Bacteroides* strains were grown on blood agar, and the colonies were evaluated for fluorescence from long-wave UV light. Most test strains of *Bacteroides melaninogenicus* subsp. *intermedius* exhibited a brilliant red fluorescence. *B. melaninogenicus* subsp. *melaninogenicus* fluoresced mostly red-orange. *Bacteroides asaccharolyticus* showed a yellow or red fluorescence. The intensity of the *Bacteroides* fluorescence weakened when the black pigment of the colonies developed. In contrast, neither young nor old colonies of the oral species *Bacteroides gingivalis* displayed fluorescence. Since *B. gingivalis* can produce severe oral infections and also can seed to nonoral sites, awareness of the inability of this organism to fluoresce is important for microbiologists utilizing UV light fluorescence to screen for black-pigmented *Bacteroides* spp. The present data also indicate that UV light fluorescence may be a rapid method of distinguishing some black-pigmented *Bacteroides* spp.

Black-pigmented *Bacteroides* spp. are commonly isolated from human mouths, intestines, and genitalia and are suspected pathogens in various mixed anaerobic infections (3). Recent taxonomic studies have identified at least nine genotypes or species of black-pigmented *Bacteroides* spp. (16). Key biochemical tests for distinguishing these bacteria by species include glucose, lactose, sucrose, and cellobiose fermentation, metabolic acid production, indole formation, esculin hydrolysis, and catalase reaction (16). Since most of these tests are broth assays, the poor growth of some black-pigmented *Bacteroides* strains in bacteriological broth media can impede classification. Another disadvantage to these tests is the extended incubation period required by several broth tests before they can be read.

Our laboratory has evaluated several rapid methods for identifying black-pigmented *Bacteroides* spp. Immunofluorescence staining appears to be potentially valuable in distinguishing these organisms by species (7, 9, 10) but the requirement for a fluorescence microscope and specific immunological reagents may limit the use of these techniques. The commercial API ZYM system (Analytab Products, Plainview, N.Y.) has differential diagnostic value (8, 15); the price and availability of this system may be limiting factors. The direct hemagglutination technique with sheep and rabbit erythrocytes can distinguish among some black-pigmented *Bacteroides* spp. (12, 17). The hemagglutination assay is simple and inexpensive, but subcultivation of the primary colony must often take place before testing to get enough bacterial cells. The cellular fatty acid profile (7) and soluble protein composition (19) are also distinguishing characteristics, but the equipment needed for these analyses may not be available in most laboratories.

Rapid screening of black-pigmented *Bacteroides* spp. has been performed by long-wave UV fluorescence (1, 4, 6, 11). The taxonomic schemes of Finegold et al. (5), Sutter et al. (18), Washington (20), and Ellner et al. (2) include red fluorescence of young colonies of *Bacteroides melaninogenicus* and *Bacteroides asaccharolyticus* as a presumptive test for these organisms. The UV fluorescence technique is appealing because of its potential for identifying black-pigmented *Bacteroides* spp. on blood agar plates even before a distinct black pigment develops. *Veillonella* spp., which also have been reported to fluoresce red (1), may be differentiated from young *Bacteroides* colonies by a Gram stain (18).

During studies of the oral microbiota in humans, however, we discovered that several colonies of black-pigmented *Bacteroides* spp. demonstrated no fluorescence with UV light. These findings prompted the present study, in which we investigated the range of black-pigmented *Bacteroides* spp. that exhibit UV fluorescence.

TABLE 1. Black-pigmented *Bacteroides* strains used in this study and results of long-wave UV fluorescence

Strain	Fluorescence after incubation[a]			Origin of strain[b]	Source[c]
	24–48 h	7 days	14 days		
B. gingivalis					
ATCC 33277 (type strain)	0	0	0	Subgingival plaque	ATCC
381, K 110	0	0	0	Subgingival plaque	S. S. Socransky
1021, 1112, 1312, 1432, PIV 5A2-8, 001, 010, 015	0	0	0	Subgingival plaque	Own isolates
B. asaccharolyticus					
ATCC 25260 (type strain)	Red (+)	0	0	Empyema	ATCC
ATCC 27067	Yellow (+)	0	0	Human leg wound	ATCC
In 4	Red (++)	Red-orange (+)	0	Unknown	H. R. Ingham
Umbilicus	Red (++)	Red-orange (+)	0	Umbilicus infection	K. J. Wicker
Blood	Yellow (+)	0	0	Bacteremia	K. J. Wicker
536 B	Red (++)	Red-orange (+)	0	Feces	V. L. Sutter
14015	Red (+)	Red-brown (+)	0	Buttock abscess	W. L. Miethaner
B. melaninogenicus subsp. *intermedius*					
ATCC 25611 (type strain)	Red-orange (++)	Pink-orange (+)	Orange (+)	Empyema	ATCC
NCTC 9336	Brilliant red (++)	Red-orange (+)	Orange (+)	Vincent's gingivitis	J. M. Hardie
ATCC 25261	Red-orange (++)	Pink-orange (+)	Orange (+)	Laryngotomy wound	ATCC
532-70 A	Brilliant red (++)	Red-orange (+)	Orange (+)	Cervical swab	D. W. Lambe, Jr.
8A2-2, 8A2-6, CC2, 4117, 4127, 20-3	Brilliant red (+++)	Red-orange (++)	Orange (+)	Subgingival plaque	Own isolates
B. melaninogenicus subsp. *melaninogenicus*					
ATCC 25845 (type strain)	Brilliant red (+++)	Orange (+)	Orange (+)	Sputum	ATCC
ATCC 33185	Yellow (++)	Yellow (+)	Yellow (+)	Maxillary atrium	ATCC
ATCC 15930	Red-orange (+)	Orange (+)	Red-brown (+)	Subgingival plaque	ATCC
DIC-20	Red-orange (++)	Orange (+)	Red-brown (+)	Subgingival plaque	L. V. Holdeman
S-2	Red-orange (++)	Orange (+)	Red-brown (+)	Monkey dental plaque	J. M. Hardie
JD-1, PIV 5A3-33, 4A3-11	Red-orange (++)	Orange (+)	Red-brown (+)	Subgingival plaque	Own isolates
B. melaninogenicus subsp. *levii*					
JP2	Pink-orange (+)	Red-brown (+)	0	Cattle horn abscess	J. M. Hardie
VPI 10458 A	Red-orange (+)	Red-brown (+)	0	Abdominal wound	L. V. Holdeman
1815, 1818	Red-orange (+)	Red-brown (+)	0	Subgingival plaque	Own isolates

[a] Fluorescence intensity, shown in parentheses, was graded as negative (0), weak (+), moderate (++), or strong (+++).

[b] All strains were isolated from humans unless otherwise designated.

[c] The strains were obtained from the following sources: ATCC, American Type Culture Collection, Rockville, Md.; S. S. Socransky, Forsyth Dental Center, Boston, Mass.; H. R. Ingham, General Hospital, Newcastle-upon-Tyne, England; K. J. Wicker, Erie County Medical Center, Buffalo, N.Y.; V. L. Sutter, Wadsworth Veterans Administration Hospital, Los Angeles, Calif.; W. L. Miethander, Buffalo General Hospital, Buffalo, N.Y.; J. M. Hardie, London Hospital Medical College, London, England; D. W. Lambe, Jr., East Tennessee State University, Johnson City, Tenn.; L. V. Holdeman, Virginia Polytechnic Institute and State University, Blacksburg, Va.

The black-pigmented *Bacteroides* strains examined are listed in Table 1. Organisms were plated on tryptic soy agar (Difco Laboratories, Detroit, Mich.) supplemented with 5% sheep blood, 5 μg of hemin per ml, and 0.2 μg of menadione per ml. The plates were incubated at 37°C in a Coy anaerobic chamber (Coy Manufacturing Co., Ann Arbor, Mich.) containing 85% N_2–10% H_2–5% CO_2. For examination of fluorescence, the plates were removed from the anaerobic chamber for approximately 15 min. The UV light source was a hand-held Mineralight lamp, model UVSL-58 with short-wave (254 nm) and long-wave (366 nm) UV light (Ultra-Violet Products, Inc., San Gabriel, Calif.). The fluorescence reaction was read in a dark room. On each day of testing, the colony color and the color and intensity of fluorescence were determined.

Table 1 lists results of long-wave UV light fluorescence for the black-pigmented *Bacteroides* strains studied. The 10 test strains of *B. melaninogenicus* subsp. *intermedius* yielded a red-orange to brilliant red fluorescence which was observable after 1 day of incubation, when the black pigment of the colonies was still indistinct, and throughout the 14-day study period. *B. melaninogenicus* subsp. *melaninogenicus* also displayed a marked fluorescence, predominantly of a red-orange hue. The fluorescence of *B. asaccharolyticus* was yellow or red. The *Bacteroides* fluorescence weakened with prolonged incubation, and for some strains it became undetectable after the development of a jet-black pigment in the colonies. These findings that strains of *B. melaninogenicus* and *B. asaccharolyticus* exhibit UV light fluorescence, although of various intensities, agree with previous fluorescence data for this group of organisms (4, 6).

A finding which has not been reported previously was the inability of *Bacteroides gingivalis* to fluoresce with UV light. This was the case when young or old colonies were examined. In addition, *B. gingivalis* did not fluoresce when the black growth was emulsified in methanol, a method which may reveal fluorescence of old cultures which otherwise have lost the capability to fluoresce (11).

Myers et al. (11) related the red fluorescence of *Bacteroides* spp. to the black pigment of the colonies. These authors showed that nonpigmenting *B. melaninogenicus* grown in the absence of blood does not fluoresce and that the red fluorescent component and porphyrins, which may cause the black pigment (14), yield similar spectral absorption curves, with peaks in the vicinity of 400 nm. If so, the present data may indicate that the black pigment of *B. gingivalis* is chemically different from that of other black-pigmented *Bacteroides* spp. That *Bacteroides* spp. can produce black pigments of various chemical natures has been shown by Reid et al. (13).

Recognition of the failure of *B. gingivalis* to fluoresce is important for clinical microbiologists who use UV light fluorescence to screen for black-pigmented *Bacteroides* spp. Since *B. gingivalis* is an oral organism, the risk for false-negative results is especially great in the study of oral microbiota. However, because *B. gingivalis* can seed to extraoral sites, the organism may also escape identification in nonoral infections. If *B. gingivalis* is suspected in a clinical specimen, reincubation for observation of black pigmentation may be necessary to identify the organism.

The difference in UV fluorescence among black-pigmented *Bacteroides* spp. may also be considered in a positive sense as being a rapid taxonomic test to distinguish between *B. gingivalis* and other species of this group of organisms.

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