Report of the ICSB Taxonomic Subcommittee on Gram-Negative Anaerobic Rods†

Proposal That the Saccharolytic and Asaccharolytic Strains at Present Classified in the Species *Bacteroides melaninogenicus* (Oliver and Wherry) Be Reclassified in Two Species as *Bacteroides melaninogenicus* and *Bacteroides asaccharolyticus*

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The asaccharolytic and saccharolytic strains at present classified together in one species, *Bacteroides melaninogenicus*, have been shown to differ in a number of properties including their deoxyribonucleic acid base ratios. It is proposed that the asaccharolytic strains (guanine-plus-cytosine content, 50 to 54 mol%) should be assigned to a separate species, *Bacteroides asaccharolyticus*, whereas the saccharolytic strains (guanine-plus-cytosine content, 40 to 45 mol%) should still be classified in the two subspecies *Bacteroides melaninogenicus* subsp. *melaninogenicus* and *Bacteroides melaninogenicus* subsp. *intermedius*, although further study is required to complete the characterization of these two groups.

For some time there has been confusion over the identity of the various black-pigmented organisms included in the species *Bacteroides melaninogenicus* first described by Oliver and Wherry in 1921 (6). Holdeman and Moore (3) recognized three subspecies: *Bacteroides melaninogenicus* subsp. *melaninogenicus*, *B. melaninogenicus* subsp. *intermedius*, and *B. melaninogenicus* subsp. *asaccharolyticus*. The ICSB Taxonomic Subcommittee on Gram-Negative Anaerobic Rods, in 1972, decided to make a special study of these organisms (4), concentrating on the properties of the American Type Culture Collection (ATCC) and National Collection of Type Cultures (NCTC) strains. A number of papers have also been published in the last 5 years which have included studies of freshly isolated strains (7, 9-11; G. M. K. Harding, V. L. Sutter, and S. M. Finegold, Abstr. Annu. Meet. Am. Soc. Microbiol., 1975, C39, p. 33; L. V. Holdeman, Round Table Session 57, Annu. Meet. Am. Soc. Microbiol., 1975). At its meeting in Bucharest in July 1975 (5), the Subcommittee made a careful assessment of all the information available and concluded that the asaccharolytic strains at present known as *B. melaninogenicus* subsp. *asaccharolyticus* should be reclassified in a separate species, *Bacteroides asaccharolyticus*.

Both the asaccharolytic and saccharolytic strains produce a black pigment that has been shown to be hematin (1). The evidence for the creation of a new species is given below.

MATERIALS AND METHODS

Cultures. The following cultures were examined: *Bacteroides melaninogenicus* subsp. *melaninogenicus* ATCC 25845 (VPI 2381) and ATCC 25847 (VPI 4196), both originally from a strain of Finegold (B 282); *Bacteroides melaninogenicus* subsp. *intermedius* ATCC 25846 (VPI 4200; originally Finegold B 485) and ATCC 25611 (VPI 4197; originally Finegold B 422); *Bacteroides melaninogenicus* subsp. *asaccharolyticus* ATCC 25260 (VPI 4198; originally Finegold B 440); *Bacteroides melaninogenicus* NCTC 9336, NCTC 9337, and NCTC 9338; and also VPI 4199 (originally Finegold B 477).

Methods. The methods were those normally used in the laboratories which participated in this study and have been published previously (2, 7-11).

RESULTS

The more important differential properties of the organisms are shown in Table 1, which includes results from 10 laboratories (Wadsworth Hospital Center, Los Angeles, Calif.; Anaerobe Laboratory, Virginia Polytechnic Institute, Blacksburg, Va.; School of Medicine, Gifu University, Japan; Animal Physiology Laboratory, The Institute of Physical and Chemical Research, Japan; Laboratoire de Bactériologie, Centre Hospitalier d'Annecy, France; Institut für Medizinische Mikrobiologie und Immunolo-

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TABLE 1. Properties of *Bacteroides melaninogenicus* subsp. *asaccharolyticus*, subsp. *intermedius*, and subsp. *melaninogenicus*^a

Property	<i>B. melaninogenicus</i> subsp. <i>asaccharolyticus</i> (now <i>B. asaccharolyticus</i>)				<i>B. melaninogenicus</i> subsp. <i>intermedius</i>			<i>B. melaninogenicus</i> subsp. <i>melaninogenicus</i>	
	NCTC 9337	ATCC 25260	VPI 4199	ATCC 25846	ATCC 25611	NCTC 9336	NCTC 9338	ATCC 25845 25847	
Formation of black colonies	+ (7) ^b	+ (5)	+ (4)	+ (5)	+ (4)	+ (5)	+ (4)	+ (4) ± or - (5)	
Fluorescence under UV light	+ (2)	+ (2)	+ (1)	+ (3)	+ (2)	+ (2) - (1)	+ (2) - (1)	+ (4)	
Difference in pH after growth in medium with and without glucose	0 (3)	0 (3)	0 (1)	1.4 (4)	0.95 (3)	1.3 (3)	0.9 (3)	1.3 (6)	
Terminal pH, glucose	6.4 (7) (6.2-6.6)	6.4 (4) (6.15-6.8)	6.3 (4)	5.2 (4) (5.0-5.4)	5.4 (3) (5.3-5.5)	4.9 (4) (4.6-5.1)	5.0 (3) (5.0-5.1)	5.1 (6) (5.0-5.3)	
Fermentation products									
Acetic acid	+ (5)	+ (4)	+ (3)	+ (3) - (1)	+ (3)	+ (3) - (1)	+ (3)	+ (6) - (1)	
Propionic acid	+ (4) - (1)	+ (4)	+ (2) - (1)	- (3) + (1)	- (2) + (1)	- (3) tr (1)	- (3)	- (7)	
Isobutyric acid	+ (4) - (1)	+ (4)	+ (2) - (1)	+ (3) tr (1)	+ (2) tr (1)	+ (2) tr (1)	- (1) tr (1) + (1)	- (4) + (3)	
n-Butyric acid	+ (5)	+ (4)	+ (3)	- (3) tr (1)	- (3)	- (3) tr (1)	- (3)	- (7) tr (1)	
Isovaleric acid	+ (5)	+ (3) - (1)	+ (3)	+ (4)	+ (3)	+ (3)	+ (1) tr (1)	+ (3) tr (2) - (1)	
Succinic acid	- (2) tr (1)	- (2) tr (2)	- (1)	+ (4)	+ (2) tr (1)	+ (3) tr (1)	+ (3)	+ (7)	
Starch hydrolysis	- (6)	- (5) var (1)	- (2) + (1)	+ (5)	+ (4)	+ (5)	+ (3) - (1)	+ (9)	
Indole	+ (5) - (2)	+ (4)	+ (4)	+ (5)	+ (4)	+ (3) var (1) - (1)	+ (3) - (1)	- (8)	
Lipase	- (3) + (1)	- (3)	- (1)	+ (2) - (1)	+ (1) tr (1) - (1)	- (3) tr (1)	+ (2)	- (5)	
Milk	D (3) nc (1) ^c	D (4)	D (2) nc (1)	D (5)	D (3)	D (1) nc (1) ACD (1)	D (2)	C (5) AC (2)	
Esculin hydrolysis	- (5)	- (3)	- (2)	- (4)	- (4)	- (4) + (1)	- (4)	+ (7) - (1)	
DNA base ratios (mol% G + C)									
A ^d	54.2	53	53	45	44	42	42	40	
B	50.0	53.8	50.7			42.7	41.2	42.2	

^a Symbols: +, positive; -, negative; tr, trace; var., variable.^b Number in parentheses indicates number of laboratories carrying out the test.^c D, digestion; A, acid; C, clot; nc, no change.^d A, B, and C are individual laboratories.

gie der Universität, Bonn-Venusberg, Germany; Institute "Dr. I. Cantacuzino" Bucharest, Romania; A.R.C. Food Research Institute, Norwich, England; Dental Bacteriology Laboratory, The London Hospital Medical College, England; Department of Bacteriology, University of Edinburgh Medical School, Scotland. Detailed studies which refer to these organisms have already been reported (3, 7, 9-11; Harding et al., Abstr. Annu. Meet. Am. Soc. Microbiol., 1975, C39, p. 33).

In some instances only a few tests were carried out or only a few strains were examined, the number of laboratories contributing to any one result being indicated in the table. Differences obtained with the same strain again demonstrates the lack of reproducibility which can occur between laboratories. In the case of the deoxyribonucleic acid (DNA) base ratios, results from individual laboratories have been recorded separately; the thermal denaturation method was used in each case.

It can be seen from Table 1 that the asaccharolytic strains are differentiated from the saccharolytic strains by their failure to produce a lower pH in the glucose medium than when grown in the same medium without glucose, whereas the saccharolytic strains had a terminal pH at least 1 pH unit lower. They can also be differentiated from the saccharolytic strains by a number of other properties. The fermentation products are different: whereas isobutyric acid is produced by both groups, *n*-butyric acid is only produced by asaccharolytic strains, and succinic acid is only produced by saccharolytic strains. Although there is variation in the results for DNA base ratios from different laboratories, the asaccharolytic strains have a guanine-plus-cytosine (G+C) content of 50 to 54 mol% whereas all of the saccharolytic strains are 40 to 45 mol% in G+C content. In addition to the results shown in Table 1, there is also some evidence of a difference in cell wall composition. The asaccharolytic strain NCTC 9337 was shown to contain lysine in the mucopeptide cell wall component, whereas the saccharolytic strains NCTC 9336 and 9338 contained diaminopimelic acid and no lysine (11).

It is evident that there are differences between the saccharolytic strains *B. melaninogenicus* subsp. *intermedius* and *B. melaninogenicus* subsp. *melaninogenicus*. Although only one strain of *B. melaninogenicus* subsp. *melaninogenicus* (ATCC 25845) is shown in the table, the results from 23 strains (9; Harding et al., Abstr. Annu. Meet. Am. Soc. Microbiol., 1975, C39, p. 33) confirm that the strains of *B. melaninogenicus* subsp. *melaninogenicus* differ in

that they hydrolyze esculin, are indole negative, produce clot in milk rather than digestion, and do not possess lipase activity.

DISCUSSION

The evidence given above indicates that organisms with quite different DNA base ratios and energy-yielding metabolism have been classified together in one species solely on the basis of their ability to produce a black pigment, hematin. It is proposed that the asaccharolytic strains of *Bacteroides melaninogenicus* should be assigned to a separate species, *Bacteroides asaccharolyticus* (type strain = ATCC 25260).

It is also proposed that the saccharolytic strains within the species *Bacteroides melaninogenicus* should still be classified in the two subspecies *Bacteroides melaninogenicus* subsp. *melaninogenicus* and *Bacteroides melaninogenicus* subsp. *intermedius*, although further study is required to complete the characterization of these two groups. It may be necessary to create further categories for other strains, e.g., Lev 1 (VPI 3300), which differ in a number of properties from the organisms shown in Table 1.

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REPRINT REQUESTS

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