Med. Microbiol. Immunol.
168, 173–177 (1980)

Medical
Microbiology and
Immunology

Evaluation of the API ZYM System for Identification of *Bacteroides* and *Fusobacterium* Species

Tor Hofstad

The Gade Institute, Department of Microbiology, University of Bergen, Bergen, Norway

Abstract. The API ZYM patterns of 97 *Bacteroides* and 25 *Fusobacterium* strains were examined. The system discriminated between *B. asaccharolyticus*, *B. melaninogenicus* ss. *melaninogenicus*, and *B. melaninogenicus* ss. *melaninogenicus*, but not between other *Bacteroides* species or between species of *Fusobacterium*. The results provided new information on the enzymatic activity of theses gorups of bacteria.

Introduction

Micromethod multitest systems for identification of anaerobic bacteria are sold commercially. Such systems are characterized by simplicity and rapidity, but cannot compete with conventional bacteriological methodology with respect to a 100 per cent correct identification of clinical isolates. The API ZYM system (API System S.A., Le Balme Les Grottes, Montalieu Vercieu, France) is a semiquantitative micromethod for detection of enzymatic activity in biological fluids and cell suspensions. The substrates are contained in a support strip, which allows contact between the enzyme and the substrate; enzymatic activity is revealed by the addition of suitable indicators.

The practical value of the API ZYM system for identification of anaerobic bacteria has been examined to a limited extent (Tharagonnet et al., 1977; Kilian, 1978; Schwan, 1979). The present report deals with the API ZYM patterns of stock cultures and clinical isolates of *Bacteroides* and *Fusobacterium* species. The study was carried out to evaluate the use of the API ZYM system for a presumptive identification of clinical isolates, and to get new information on the enzymatic properties of this group of anaerobic organisms.

Offprint requests to: Dr. Tor Hofstad, Mikrobiologisk avdeling, MFH-bygget, N-5016 Haukeland sykehus, Norway

0300–8584/80/0168/0173/$ 01.00

Materials and Methods

Bacterial Strains. The strains tested are listed in Table 1. The clinical isolates originated from pus (*B. fragilis* and related species) or from the gingival crevice region of human adults with periodontal disease (*B. melaninogenicus* and *F. nucleatum).* They were identified according to the Wadsworth Anaerobic Bacteriology Manual (Sutter et al., 1975).

Table 1. Test strains

Bacteroides	
B. fragilis	NCTC 9343, E323 (H. Beerens, Lille, France), SBL B55, SBL B59, 23 clinical isolates
B. distasonis	ATCC 8503, 10 clincical isolates
B. ovatus	6 clinical isolates
B. thetaiotaomicron	ATCC 12290, VPI 5333, 6 clinical isolates
B. vulgatus	ATCC 8482, SBL B72, 4 clinical isolates
B. asaccharolyticus	ATCC 25260, NCTC 9337, VPI 4199
B. melaninogenicus	
ss. *intermedius*	NCTC 9336, VPI 7791-C, VPI 4197=ATCC 25611, VPI 4206 9 clinical isolates
ss. *melaninogenicus*	VPI 4196, VPI 7570 A, 30, VPI 5328, 6 clinical isolates
ss. *levii*	BM 78-30, BM 78-54, BM 10450 (G. Høi-Sørensen, Copenhagen, Denmark)
B. disiens	VPI 8057, VPI 7852
B. bivius	VPI 6822, VPI 6318
B. ruminicola	C12 and D46 (M.P. Bryant, Urbana, Ill., USA)
B. praeacutus	VPI 0217-1
B. putridinis	VPI 4998-1
B. serpens	VPI 0950-1
B. splanchnicus	Brüssel 1 (H. Werner, Bonn, FRG)
Fusobacterium	
F. nucleatum	ATCC 10953, ATCC 25586, Fev 1 (S. Mergenhagen, Bethesda, Md., USA 6 clinical isolates
F. necrophorum	VPI 6161, N167 Fievez, SPH 1 (T. Justesen, Copenhagen, Denmark)
F. mortiferum	VPI 4249, VPI 5696, VPI 0473, VPI 4123A
F. gonidiaformans	VPI 4381, VPI 0482A, VPI 4879, VPI 11360
F. varium	ATCC 8501, VPI 4234, VPI 499A
F. necrogenes	VPI 2668 = ATCC 25566

ATCC = American Type Culture Collection, Rockville, Md., USA
NCTC = National Collection of Type Cultures, Central Public Health Laboratory, London, England
SBL = National Bacteriological Laboratory, Stockholm, Sweden
VPI = Virginia Polytechnic Institute and State University, Blacksburg, Va., USA

API ZYM Tests. Each strain tested was grown anaerobically (GasPak system) on Brucella blood agar plates (7 per cent human blood) for 48 h at 37°C. The growth from one, occasionally two plates was carefully removed from the surface of the plate, and suspended in 2 ml of sterile saline to give a dense suspension (turbidity higher than MacFarland standard No. 5). Two drops of the suspension were added with a Pasteur pipette to each of the 20 cupules of the API ZYM system strip. Each strip was placed in a moist chamber, and incubated aerobically for 4 h at 37°C. One drop of each of API developing reagents A [Tris (hydroxymethyl) aminomethane 250 g, HCl 37 per cent 110 ml, laurylsulphate 100 g, distilled water to 1 l] and B (Fast Blue BB 3.5 g, 2-methoxyethanol to 1 l) was added to each of the cupules, and the intensity of the colour reactions which developed within 5 min was graded from zero to 5 with reference to a API ZYM colour reaction chart. All stock strains were examined at least twice.

Results

The reactions of the strains tested for acid phosphatase, phosphoamidase, and the glycosidases included in the API ZYM test system are listed in Table 2. The reactions were consistent on repeated testing. Ten strains labelled as *B. oralis* had the same patterns of positive reactions as the *B. melaninogenicus* ss. *melaninogenicus* strains. As the validty of the species *B. oralis* is under discussion (ICSB Sub-Committee on Gram-Negative Anaerobic Rods) these strains are not listed in Table 1 and Table 2. All *Bacteroides* strains and the strains of *F. mortiferum* gave strong positive reactions (grade 5 or 4) for alkaline phosphatase. The remaining *Fusobacterium* strains reacted weakly (grade 1) in the test system for this enzyme. Most strains reacted weakly in the test systems for esterase and esterase lipase. However, wash waters from uninoculated blood agar plates sometimes produced weak reactions in the same systems. None of the strains examined gave reactions with the substrates for lipase, leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin and chymotrypsin, which were consistent on repeated testings.

Discussion

The results show that the API ZYM system does not differentiate between *B. fragilis* and the related, clinically less important species within the fragilis group, *viz. B. distasonis, B. ovatus, B. thetaiotaomicron,* and *B. vulgatus.* Nor does the system differentiate between the species of *Fusobacterium* most often found in clinical material. *F. mortiferum* may be an exception. The value of the test system for identification of anaerobic Gram-negative non-sporeforming rods seems to be limited to the distinction between *B. asaccharolyticus, B. melaninogenicus* ss. *intermedius,* and *B. melaninogenicus* ss. *melaninogenicus.* The system might be of some use for differentiation of penicillin resistant strains of *B. disiens* and *B. bivius* from strains within the *fragilis* group of organisms.

Natural substrates for α-galactosidase and β-galactosidase are melibiose and raffinose, and lactose, respectively. Maltose is substrate for α-glucosidase and cellobiose for β-glucosidase. The positive reactions of the saccharolytic *Bacteroides* for these enzymes agree with their fermentation reactions (Holdeman et al., 1977).

Table 2. Reactions of *Bacteroides* and *Fusobacterium* strains in the API ZYM test systems for acid phosphatase, phosphoamidase, and glycosidases

Bacterial species	No. of Strains	No. of strains reacting with test system for[a] acid phosphatase 11	phospho-amidase 12	α-galacto-sidase 13	β-galacto-sidase 14	β-glucuro-nidase 15	α-glucosi-dase 16	β-glucosi-dase 17	β-N-acetyl-glucosaminidase 18	α-fucosi-dase 20
Bacteroides	97									
B. fragilis	27	27	27	26	27	27	27	(4)	27	27
B. distasonis	11	11	11	11	11	6	10	4	11	6
B. ovatus	6	6	6	6	6	5	6	1	6	6
B. thetaiotaomicron	8	8	8	8	8	5	6	4	6	6
B. vulgatus	6	6	6	6	6	6	6	0	6	4
B. asaccharolyticus	3	3	3	0	0	0	0	0	0	2
B. melaninogenicus										
ss. intermedius	13	13	13	0	0	0	13	0	0	12
ss. melaninogenicus	10	10	10	7	10	1	10	0	10	10
ss. levii	3	3	3	0	0	0	0	0	3	0
B. disiens	2	2	2	0	0	0	2	0	0	0
B. bivius	2	2	2	0	2	0	2	0	2	2
B. ruminicola	2	2	2	2	2	0	2	2	2	0
B. praeacutus	1	1	1	0	0	0	0	0	0	0
B. putredinis	1	1	1	0	0	0	0	0	0	1
B. serpens	1	1	1	1	1	(1)	1	1	1	1
B. splanchnicus	1	0	0	1	(1)	0	(1)	0	0	1
Fusobacterium	25									
F. nucleatum	9	(8)	(5)	0	0	0	0	0	0	0
F. necrophorum	3	3	2	0	0	0	0	0	0	0
F. mortiferum	4	4	4	(4)	(4)	0	0	0	0	0
F. gonidiaformans	4	(1)	(1)	0	0	0	0	0	0	0
F. varium	4	4	0	0	0	0	0	0	0	0
F. necrogenes	1	1	0	0	0	0	0	0	0	0

a Composition of test substrates: 11 = 2 naphtyl phosphate, 12 = naphtol-AS-B1-phospho-diamide, 13 = 6-Br-2-naphtyl-αD-galacto-pyranoside, 14 = 2-naphtyl-βD-galactopyranoside, 15 = naphtol-AS-B1-βD-glucuronate, 16 = 2-naphtyl-αD-glucopyranoside, 17 = 6Br-2-naphtyl-βD-glucopyranoside, 18 = 1-naphtyl-N-acetyl-βD-glucosaminide, 20 = 2-naphtyl-αL-fucopyranoside. ( ) grade 1 reaction only. None of the strains reacted with the substrate for α-mannosidase

The reactions of the strains tested with the substrates for β-glucuronidase, β-N-acetylglucosaminidase, and α-fucosidase yielded new information about the bacterial species tested. Nearly all strains within the *fragilis* group gave a positive reaction for β-glucuronidase, and, with a few exceptions, all saccharolytic *Bacteroides* strains reacted with the test systems for β-N-acetylglucosaminidase and α-fucosidase. β-glucuronidase attacks β-D-glucopyranosideuronates, which may be present in several polysaccharides. β-N-acetylglucosaminidase catalyses glucosaminide linkages in hyaluronic acid degradation products and has the potential of splitting off terminal N-acetylglucosamine residues. Natural glycoproteins having α-linked fucose as a terminal sugar are potential substrates for α-fucosidase. These enzymes may be involved in the breakdown of tissue taking place in pyogenic infections caused by *B. fragilis* and the other saccharolytic *Bacteroides* species.

References

Kilian, M.: Rapid identification of *Actinomycetaceae* and related bacteria. J. Clin. Microbiol. **8**, 127–133 (1978)

Holdeman, L.V., Cato, E.P., Moore, W.E.C.: Anaerobe Laboratory Manual, 4 ed., Blacksburg, Va., Anaerobe Laboratory, Virginia Polytechnic Institute and State University 1977

Schwan, O.: Biochemical, enzymatic, and serological differentation of *Peptococcus indolicus* (Christiansen) Sørensen from *Peptococcus asaccharolyticus* (Distaso) Douglas. J. Clin. Microbiol. **9**, 157–162 (1979)

Sutter, V.L., Vargo, V.L., Finegold, S.M.: Wadsworth Anaerobic Bacteriology Manual, 2 ed. Los Angeles, Calif. Anaerobic Bacteriology Laboratory, Wadsworth Hospital and Department of Medicine, UCLA 1975

Tharagonnet, D., Sisson, P.R., Roxby, C.M., Ingham, H.R., Selkon, J.B.: The API Zym system in the identification of Gram-negative anaerobes. J. Clin. Pathol. **30**, 505–509 (1977)

Received January 27, 1980