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Extracellular Enzymes of the Genus *Bacteroides*

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The extracellular production of hyaluronidase and chondroitin sulfatase was demonstrated in all of the subspecies of *Bacteroides fragilis* tested with the exception of *B. fragilis* subsp. *vulgatus*. Elastase was found only in one strain of *B. coagulans* tested. This appears to be the first report of these enzyme activities in this genus. Additional enzymes found to be produced by certain members of this genus were fibrinolysin, penicillinase, lysozyme, lecithinase, deoxyribonuclease, phosphatase, protease, and lipase.

The full complement of extracellular enzymes elaborated by the genus *Bacteroides* is not known. Heparinase (5), collagenase (6), fibrinolysin (20), and neuraminidase (10) have been reported for some species. Recently, the extracellular production of phosphatase (14) and deoxyribonuclease (DNase) (13) was reported. Certain species of *Bacteroides* also produce lipase (7) and protease (19). The purpose of this investigation was to determine whether biologically active substances other than those reported for this genus are also produced.

Thirty-three organisms (15 species or subspecies of the genus *Bacteroides*) were obtained from three sources: the University of Illinois Anaerobe Laboratory, Chicago; Anaerobe Laboratory at the Center for Disease Control, Atlanta, Ga.; and the Anaerobe Laboratory of the Virginia Polytechnic Institute (VPI) and State University, Blacksburg. Before use, all cultures were recharacterized by determining whether the cultures fermented arabinose, cellobiose, fructose, glucose, lactose, maltose, mannitol, mannose, raffinose, rhamnose, sucrose, trehalose, and xylose, reduced nitrate, hydrolyzed esculin, produced indole, and were able to grow in media containing 20% bile. The tests were performed by inoculating the cultures in appropriate prereduced (Scott Laboratories, Fiskeville, R.I.) or thioglycolate-base media and incubating the cultures at 37°C for 48 h in an anaerobic glove box (Coy Manufacturing Co.). The patterns of sugar fermentations and of other biochemical tests were then compared with specific charts for bacteroides in the VPI manual (7). The species and subspecies of these organisms were thus determined. This was further confirmed by gas chromatography of volatile and nonvolatile acids following the methods listed in the VPI manual. Seven of the 40 organisms tested did not match their original identification and were thus omitted from the study.

The bacteria were tested for the production of fibrinolysin (1), elastase (11), penicillinase (12), lysozyme (18), lecithinase (9), esterase (9), lipase (9), hyaluronidase (17), chondroitin sulfatase (17), protease (8), DNase (13), and phosphatase (2). All substrate plates were prepared according to the procedures described by previous investigators with the following exceptions: (i) brain heart infusion (BHI) supplemented with 0.5% yeast extract constituted the basal media; (ii) all media excluding those for DNase and phosphatase were solidified with the addition of 1.0% agarose (Colab); (iii) DNase agar (Difco) was prepared according to the manufacturer's directions; (iv) the medium for the phosphatase test contained 3% agar; and (v) production of penicillinase was detected by incorporating penicillin (10^3 U/ml) in BHI agarose plates containing 1% starch.

Before testing, the organisms were grown on BHI blood agar plates for 48 to 72 h in the anaerobic glove box at 37°C. The gas mixture was 10% CO_2, 10% H_2, and the balance N_2. Colonies were transferred via stab inoculations to the various substrate plates. The plates were incubated for 72 h at 37°C in the anaerobic environment. Each assay was performed in duplicate. All reactions with the exception of the penicillinase production were developed and interpreted as described by the original investigators. In the case of penicillinase production, the cultures could be separated as penicillin sensitive or penicillin resistant on the basis of growth after incubation of the penicillin plates. Presence of penicillinase was then detected by flooding the plate with Gram iodine. This procedure yielded a clear zone around colonies where penicillin had been hydrolyzed to penicilloic acid. No zones were evident on plates con-

TABLE 1. *Some extracellular enzymes of selected species of the genus Bacteroides*

Organism[a]	No. of strains	Enzyme[b]											
		FIB	ELA	PEN	LYS	LEC	EST	HYA	CHON	DNase	PHOS	PRO	LIP
B. fragilis subsp. *fragilis* (UI)	6	−	−	+	−	−	−	$+^{w}$	+	+	+	+	$+^{-}$
B. fragilis subsp. *thetaiotaomicron* (CDC 15438,17548)	2	−	−	ND	−	−	−	+	+	+	+	−	−
B. fragilis subsp. *ovatus* (UI and CDC 15866)	2	−	−	+	−	−	−	+	+	+	+	−	−
B. fragilis subsp. *distasonis* (UI and CDC 15756)	5	−	−	$+^{-}$	−	−	−	+	+	+	+	$+^{-}$	−
B. fragilis subsp. *vulgatus* (UI and CDC 17792)	3	−	−	−	−	−	−	−	−	+	+	+	−
B. melaninogenicus subsp. *asaccharolyticus* (UI and CDC 18035)	2	+	−	−	−	−	−	−	−	+	+	+	+
B. melaninogenicus subsp. *intermedius* (UI and CDC 17787)	2	$+^{-}$	−	−	−	−	−	−	−	+	+	+	+
B. oralis (UI)	2	−	−	+	w	+	−	−	−	−	+	+	+
B. ruminicola subsp. *brevis* (VPI 0050-2 and UI)	3	−	−	+	−	−	−	−	−	+	+	−	−
B. clostridiiformis subsp. *clostridiiformis* (UI)	1	−	−	−	−	−	−	−	−	+	+	+	+
B. putredinis (VPI 7437)	1	+	−	−	w	+	−	−	−	−	−	+	+
B. capillosus (VPI 8788)	1	−	−	+	−	−	−	−	−	+	+	−	+
B. coagulans (VPI 6453)	1	−	+	−	−	−	−	−	−	−	−	+	+
B. ochraceus (CDC 2845-2)	1	−	−	+	−	−	−	−	−	+	+	−	−
B. furcosus (CDC 3253)	1	−	−	+	−	−	−	−	−	−	+	−	−

[a] Abbreviations: UI, University of Illinois Anaerobe Laboratory; CDC, Center for Disease Control; VPI, Virginia Polytechnic Institute and State University.

[b] Abbreviations: FIB, Fibrinolysin: ELA, elastase; PEN, penicillinase; LYS, lysozyme; LEC, lecithinase; EST, esterase; HYA, hyaluronidase; CHON, chondroitin sulfatase; DNase, deoxyribonuclease; PHOS, phosphatase; PRO, protease; LIP, lipase. +, Moderate to strong reaction; −, no reaction; w, weak reaction; $+^{-}$, majority positive, occasional negative; $+^{w}$, majority positive, occasional weak; ND, not done.

taining only penicillin-sensitive organisms.

In addition to the enzymes already reported in the literature, elastase, hyaluronidase, and chondroitin sulfatase were found to be produced by some of the organisms tested (Table 1). Hyaluronidase and chondroitin sulfatase were found in the strains of *B. fragilis* subsp. *fragilis*, *B. fragilis* subsp. *distasonis*, *B. fragilis* subsp. *ovatus*, and *B. fragilis* subsp. *thetaiotaomicron* tested. Elastase was found only in the one strain of *B. coagulans* tested.

As far as we can ascertain, this is the first time that these extracellular hydrolase activities have been reported for this group. Previously Gesner and Jenkin (5) reported hyaluronidase and chondroitin sulfatase activity from a culture identified as *Bacteroides*. However, in their investigation, the enzymes were detected only after grinding the organism and thus appeared to be intracellular.

The role of these enzymes in an infectious process is uncertain. These hydrolases are produced by some of the common as well as uncommon isolates of *Bacteroides*. In other bacteria, the role of these enzymes in an infectious process has been suggested as an invasive or spreading factor (3, 4, 11, 15, 16). In *Bacteroides* this role may be the same. However, in vivo experiments should be conducted to elucidate the production and action of such enzymes in experimental animals.

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