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Extracellular Deoxyribonuclease Production by Anaerobic Bacteria

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The production of extracellular deoxyribonuclease was examined with anaerobic organisms isolated from clinical specimens. Nuclease activity was extraordinarily common. All strains of *Fusobacterium*, including eight species, as well as *Bacteroides fragilis* and *B. melaninogenicus*, displayed enzyme activity. Whereas the gram-positive bacteria were generally less productive, all strains of *Clostridium perfringens*, *Peptostreptococcus intermedius*, and *P. anaerobius* specifically produced deoxyribonuclease. The test is taxonomically valuable, particularly in the characterization of gram-positive cocci, since a deoxyribonuclease-producing coccus indicates *P. intermedius* or *P. anaerobius*. Additionally, possession of the enzyme may prove to be a useful correlate of the potential pathogenicity of anaerobes.

Extracellular deoxyribonuclease (DNase) production has been examined with many microorganisms. Its significance has been discussed in relation to pathogenicity and in reference to taxonomy. It has been established that DNase correlates with coagulase production in the identification of *Staphylococcus aureus* (4, 13), but is not necessarily indicative of potential pathogenicity of the organism (7). The armamentarium of extracellular enzymes associated with *Pseudomonas aeruginosa* and *Streptococcus pyogenes* growth has been shown to include DNase (1, 6, 11). The differentiation of *Serratia* from *Enterobacter* species (9) relies in part on detection of the enzyme. Additionally, the ability to produce DNase is indicative of several yeast genera (2).

This study concerns the ability of anaerobes to produce extracellular DNase. A variety of organisms obtained from clinical specimens are evaluated by different methods for assay of DNase activity (5, 9, 10). The application of nuclease production to the taxonomy of anaerobes and its possible role as a correlate of pathogenicity is discussed.

MATERIALS AND METHODS

Test organisms. Stock cultures and recent isolates from clinical specimens were employed in the study. All organisms were identified in the Anaerobic Laboratory at Temple University School of Medicine by performing appropriate biochemical tests and gas chromatography analysis as described by the Anaerobic Laboratory of the Virginia Polytechnic Institute and State University (3). Additional strains were provided by the Anaerobic Laboratory at the Center for Disease Control.

Media. The production of DNase was determined on several different media. DNase test agar (BBL) was supplemented with 0.3% yeast extract. Brucella and brain heart infusion agar (BBL) were supplemented with 2 mg of nucleic acid per ml (bull sperm deoxyribonucleic acid [DNA]; Nutritional Biochemicals Corp., Cleveland, Ohio) and 0.3% yeast extract. The modifications of the DNase test in which toluidine blue (9) or methyl green (10) dyes are incorporated into the medium were also evaluated. The commercial DNase test agar was the basal medium employed in this study.

Test procedures. Agar plates were prepared with 20 ml of media. This provided a suitable depth of medium which afforded accurate readings with weak enzyme-producing organisms. The medium was stored aerobically at 4 C and was not used after 7 days. Colonies picked from 48-h cultures on blood agar plates were streaked onto the agar surface, incubated, and then tested by flooding the plates with 1 N HCl (5). Production of DNase results in hydrolysis of the DNA into nucleotide fractions which are not precipitated by acid, this being demonstrated by a clear zone surrounding the DNase-producing colonies, while the unattacked DNA forms a cloudy precipitate in the medium. Several organisms were tested on a single plate. Cultures were incubated at 37 C for a minimum of 48 h or until adequate growth was obtained (5-day maximum). Organisms were grown in GasPak jars using the evacuation-replacement method (flushed three times) to provide an atmosphere of 85% N_2, 10% H_2, and 5% CO_2.

RESULTS

Fortified media (brucella, brain heart infusion, and Trypticase soy bases) containing DNA were tested for their ability to support growth of anaerobes and indicate DNase activity. Since little difference was observed among the media, the commercial DNase test medium was employed throughout the study.

Addition of dyes to the medium has made possible the determination of enzyme activity without flooding the plates with hydrochloride and killing the organisms. DNA hydrolysis is indicated by clearing of the medium containing methyl green or by production of pink zones in toluidine blue medium. However, under anaerobic conditions this indicator system was nullified since clearing of the medium containing either dye occurred upon growth of all organisms. The technique was abandoned because of this.

The production of extracellular DNase was a common characteristic with many anaerobic bacteria. Table 1 shows the enzyme activity of gram-negative organisms after 72 h of incubation. The three genera of organisms examined were *Bacteroides*, *Fusobacterium*, and *Veillonella*. All 24 strains of *Fusobacterium*, representing eight species, produced the exoenzyme. The *Bacteroides* species which displayed specific nuclease activity included *B. fragilis* and *B. melaninogenicus*, while three of four strains of *B. ruminicola* and the one strain of *B. clostridiiformis* were also active. Species showing no enzyme activity were *B. corrodens* and *B. pneumosintes*. One of three strains of *Veillonella* displayed enzyme activity.

The production of DNase was also a property common to many gram-positive anaerobes (Table 2). The three *Clostridium* species examined (five of the seven strains were *C. perfringens*) displayed nuclease activity, whereas nonsporeforming rods were generally nonactive. Only one of eight strains of *Bifidobacterium* and three of 13 strains of *Eubacterium* species hydrolyzed DNA. It should be mentioned that, of the few strains of *Eubacterium* speciated, two strains identified as *E. cylindroides* were both nuclease producers.

The results of gram-positive cocci were significant, since a considerable number of strains was tested for most species and specific characteristics were demonstrated. All 13 strains of *Peptostreptococcus anaerobius* and the five strains of *P. intermedius* hydrolyzed DNA. The activity of *P. anaerobius* was typically weak but always discernible. The cocci which did not demonstrate an active nuclease included *P. micros*, *Peptococcus magnus*, *P. asaccharolyticus* (one exception), *P. prevotii*, and *Gaffkya anaerobia*.

TABLE 1. *Production of extracellular DNase by gram-negative anaerobes*[a]

Organism	No. of strains	
	Total	Producing DNase
Bacteroides fragilis	29	28
B. melaninogenicus	10	10
B. ruminicola	4	3
B. corrodens	3	0
B. pneumosintes	2	0
B. clostridiiformis	1	1
Fusobacterium symbiosum	3	3
F. nucleatum	2	2
F. gonidiaformans	4	4
F. mortiferum	9	9
F. naviforme	1	1
F. russii	1	1
F. necrophorum	1	1
F. varium	3	3
Veillonella parvula	2	1
V. adolescens	1	1

[a] Cultures were incubated at 37 C for 72 h.

DISCUSSION

Since many anaerobes have fastidious growth requirements, the selection of a suitable test medium was essential. The fortified commercial DNase test medium was selected for convenience, since the other media did not offer any advantages.

TABLE 2. *Production of extracellular DNase by gram-positive anaerobes*[a]

Organism	No. of strains	
	Total	Producing DNase
Peptococcus magnus	10	0
P. asaccharolyticus	12	1
P. prevotii	13	0
Peptostreptococcus micros	4	0
P. anaerobius	13	13
P. intermedius	5	5
Gaffkya anaerobia	2	0
Clostridium perfringens	5	5
C. plagarum	1	1
C. putrificum	1	1
Eubacterium species	13	3
Bifidobacterium species	8	1
Lactobacillus species	2	0
Propionibacterium acnes	4	0

[a] Cultures were incubated at 37 C for 72 h.

The reactions were recorded after 48- and 72-h incubation periods. Some organisms displayed delayed DNase production which was detected only after 72 h. This was particularly observed with several strains of fusobacteria and veillonellae and was believed to be attributed to their slow growth rate. Examination of organisms was additionally performed after 5 days; however, no new nuclease-producing organisms were demonstrated.

The hydrochloride flooding technique gave satisfactory results. Streaks of growth were scraped from the medium before reading the reactions. This facilitated the detection of slight nucleic acid hydrolysis.

Among the gram-negative organisms, extracellular nuclease production was extraordinarily common. All the strains of *Fusobacterium* and the clinically significant *Bacteroides* species, *B. fragilis* and *B. melaninogenicus*, were enzymatically active.

Whereas DNase production was less common among gram-positive bacteria, the common clinical isolates, *C. perfringens*, *P. anaerobius*, and *P. intermedius*, displayed specific nuclease activity. It is generally considered that anaerobic cocci, which are frequently isolated along with other known pathogens from human infections, do not contribute significantly to the infection but are common commensals. Nevertheless, a better understanding of the biochemical and physiological properties of anaerobic cocci is needed. It may be important that the production of DNase and other enzymes (9) is characteristic of particular strains.

The use of DNase production in classification has been common with many aerobic organisms. The information obtained with anaerobic bacteria shows that detection of nuclease activity can be valuable in the identification of certain organisms. Both *B. corrodens* and *B. pneumosintes* lacked DNase activity; however, the number of strains tested was not significant. The enzyme was specifically produced by *P. anaerobius* and *P. intermedius*, whereas the other cocci failed to demonstrate this property. Furthermore, since *P. anaerobius* displayed weak hydrolysis and *P. intermedius* displayed much stronger activity, this may provide a simple method of differentiating the two species.

In conclusion, we have described a simple method by which extracellular DNase production was determined for commonly isolated anaerobes. This represents the first report describing nuclease activity by these organisms. Generally, nuclease production was widely distributed among anaerobes. The evidence reveals that the more frequently isolated pathogens, *B. fragilis*, *B. melaninogenicus*, and *C. perfringens* produce an active enzyme. On the other hand, certain species of anaerobic gram-positive cocci produce nuclease. Although their significance in pathogenicity is unclear, in certain cases the cocci have been implicated as the major cause of infection (12). Thus the correlation of nuclease production and potential pathogenicity needs further study.

Additionally, production of DNase can be employed taxonomically. Nuclease activity among gram-positive cocci indicates either *P. anaerobius* or *P. intermedius*. Thus, this simple test should aid in the speciation of anaerobic cocci.

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