Production of Deoxyribonuclease, Ribonuclease, Coagulase, and Hemolysins by Anaerobic Gram-Positive Cocci

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Clinical isolates of *Peptococcus* and *Peptostreptococcus* species and *Streptococcus intermedius* strains were obtained from local hospitals. After confirmed identification, each isolate was tested for the in vitro production of deoxyribonuclease, ribonuclease, coagulase, and hemolysins. Of the 60 strains studied, 18 had enzymatic activity. The variability of enzyme production suggests that such assays are not suitable as an aid to identification of these organisms.

The ability of an organism to produce extracellular enzymes is frequently used to assist identification and evaluation of its clinical significance. However, there are few reports on extracellular enzyme production by anaerobic gram-positive cocci. Porschen and Sonntag (3) observed that 19 of 57 isolates produced deoxyribonuclease (DNase). They suggested that DNase production might be a useful criterion in taxonomy as well as in the assessment of the virulence of isolates. Recent studies with *Peptococcus indolicus* have shown that strains isolated from cases of bovine summer mastitis produced DNase, ribonuclease (RNase), coagulase, and hemolysins (4-7). Additionally, coagulase was produced by a strain of *P. indolicus* isolated from a human wound infection (1).

We report here our examination of 65 strains of anaerobic gram-positive cocci with respect to their production of extracellular enzymes. Sixty of the strains were recent clinical isolates obtained from local hospitals. Five were reference strains: *P. indolicus* SUAS 322, SUAS 327, SUAS 14777, and VPI 12694, and *Peptococcus magnus* WVA 2508. The SUAS strains were obtained from the Swedish University of Agricultural Sciences, the VPI strain was obtained from the Virginia Polytechnic Institute, and the WVA strain came from the Wadsworth Veterans Administration Hospital, Los Angeles, Calif.

All strains were checked for purity and hemolytic activity by inoculation onto blood agar medium (Columbia blood agar base, BBL Microbiology Systems, Cockeysville, Md., with 5% sheep blood) and anaerobic incubation by the disposable GasPak system (BBL Microbiology Systems) at 37°C for 48 h or until adequate growth was obtained (5 days maximum). Standard methods (3) including biochemical tests and gas chromatography were used to confirm the

identity of all strains. Tests for DNase were made according to Porschen and Sonntag (3) on DNase test medium without dye (BBL Microbiology Systems). Tests for RNase were made on brain heart infusion agar (BBL Microbiology Systems) containing 0.4% ribonucleic acid from *Torula* yeast (Calbiochem, La Jolla, Calif.). Inoculation, incubation, and subsequent detection of RNase by flooding with 1 N hydrochloric acid were as for the DNase plates. Clotting of rabbit plasma (Difco Laboratories, Detroit, Mich.) after 3 h of incubation at 37°C indicated a positive coagulase test.

Table 1 shows that of 60 strains isolated from a wide variety of clinical specimens, 18 produced one or more of the enzymes studied. We found that only two of eight strains of *Peptostreptococcus anaerobius* produced DNase, a result which differs from the previous study (3) in which all isolates of this strain produced the enzyme. In addition, we found a greater percentage of nuclease-positive *Peptococcus asaccharolyticus* and *Peptococcus prevotii* strains: 60% and 31% as compared to 8% and 0%, respectively, in the earlier study. Only 3 of 60 test isolates in our investigation produced hemolysins; activity was lost after subculture of the organisms onto a second blood agar plate. We further observed that enzymatic activity was more readily detected when the isolates were subcultured onto the appropriate test medium from blood agar. Subcultures of enzymatically active isolates from cooked-meat broth (Difco) showed little or no activity. In no case was a positive result obtained for any enzymatic test by increasing incubation time after productive growth was evident.

Whereas the results of this study indicate that some clinical isolates of anaerobic gram-positive cocci produce extracellular enzymes in vitro, the

TABLE 1. Extracellular enzyme production by anaerobic gram-positive cocci

Strain ^a	Clinical source	No. of strains					
		Total	Posi- tive ^b	Producing			
				DNase	RNase	Coagu- lase	He- moly- sins
Clinical isolates							
<i>Peptococcus magnus</i>	Cysts, abscesses, dental specimens	20	0	0	0	0	0
<i>P. asaccharolyticus</i>	Wounds, abscesses	10	6	5	3	0	2
<i>P. prevotii</i>	Abscesses, drainage, aspirations	16	5	5	1	0	0
<i>Peptostreptococcus anaerobius</i>	Abscesses, genital tract infections	8	3	2	2	0	0
<i>Streptococcus interme- dius</i>	Pleural fluid, peritoneal fluid, drainage	6	4	3	4	0	1
Reference strains							
<i>Peptococcus indolicus</i> (SUAS 322, 327, 14777) ^b	Bovine mastitis	3	3	3	2	3	2
<i>P. indolicus</i> (VPI 12694)	Human wound	1	1	0	0	1	0
<i>P. magnus</i> (WVA 2508)	Abdominal wound drainage	1	0	0	0	0	0

^a SUAS, Swedish University of Agricultural Sciences; VPI, Virginia Polytechnic Institute; WVA, Wadsworth Veterans Administration Hospital.

^b Number includes organisms producing one or more of the enzymes.

inconsistency of production within species would clearly lead to ambiguous results if this criterion were used as an aid to identification of isolates in the clinical laboratory. Although the production of extracellular enzymes is generally correlated with the virulence of the isolate, the existence of such an association for anaerobic gram-positive cocci has yet to be shown.

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