

Sodium Polyanethol Sulfonate Sensitivity of Anaerobic Cocci

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Growth of *Peptostreptococcus anaerobius* was shown to be totally inhibited by sodium polyanethol sulfonate (SPS). Other anaerobic cocci grew in the presence of SPS although some strains of *Peptococcus prevotii* and *Peptococcus magnus* showed delayed growth. A SPS disk assay for the presumptive identification of *P. anaerobius* is described.

Sodium polyanethol sulfonate (SPS) is a synthetic, heat stable, anticoagulant which inhibits antibacterial agents present in blood. The ability of SPS to inhibit also the growth of anaerobic cocci has been known for several years (5). Recently, SPS was shown to inhibit 7 of 16 clinical isolates of *Peptostreptococcus* but it did not inhibit any of 17 clinical isolated of *Peptococcus* (F. E. Kocka et al., Amer. J. Clin. Pathol., in press). The present study was initiated to investigate whether a particular species of *Peptostreptococcus* is inhibited by SPS. As a result of this study, a simple disk assay was developed as a presumptive test for *Peptostreptococcus anaerobius*.

MATERIALS AND METHODS

Bacterial cultures. The organisms were either clinical isolates from the University of Chicago Hospitals and Clinics or stock cultures received from V. R. Dowell of the Center for Disease Control, Atlanta, Ga. Anaerobic bacteria were identified by the methods described in the V.P.I. Anaerobe Laboratory Manual (6).

Media. Dehydrated culture media were obtained from either Wilson Diagnostic Inc. (Glenwood, Ill.) or BBL division of Bioquest (Cockeysville, Md.). Media were preduced by the methods described by Holdeman and Moore (6). SPS was purchased from Roche Diagnostics (Nutley, N.J.) and a 5% aqueous solution (Grobax).

SPS tube assay. Organisms were tested for growth inhibition in preduced brain heart infusion broth and in thioglycolate medium, both of which contained 0.05% SPS. Comparisons of growth in the tubes were made after 7 days at 37 C with a control culture without SPS.

Disk assay. Sterile blank concentration disks (¼") were obtained from Difco Laboratories (Detroit, Mich.). A 20-μliter amount of 5% SPS was added to each disk. The disks were then dried at room temperature. Test organisms were heavily streaked on Schaedler-blood agar plates, and the disks were

placed on the inoculum. After 48 h of incubation at 37 C in a Gas Pak jar (Bioquest, Cockeysville, Md.), the plates were examined for growth inhibition by SPS. Optochin and bacitracin disks were obtained from BBL division of Bioquest and used per manufacturer's directions.

RESULTS

Growth of cultures of *Peptostreptococcus anaerobius* was inhibited by SPS in concentrations ranging from 0.025 to 0.05% (wt/vol) in thioglycolate or brain heart infusion medium. Two cultures of *Peptococcus prevotii* and one culture of *Peptococcus magnus* showed delayed growth whenever SPS was present; however, they did eventually grow. All other anaerobic cocci grew well in the presence of SPS (Table 1).

Growth of all 27 strains of *P. anaerobius* was inhibited by the SPS disks (Fig. 1). The diameter of the zones of inhibition of *P. anaerobius* was 12 to 14 mm and their margins were distinct, but there was no zone of inhibition around the other anaerobic cocci (Fig. 2). Some cultures of *P. prevotii* and *P. magnus* showed a decreased amount of growth around the disk but the growth was clearly visible (Fig. 2).

Cultures of aerobic cocci shown to be sensitive to bacitracin and optochin were tested with the SPS disk. None of eight cultures of *Streptococcus pneumoniae* were sensitive, nor were eight cultures of *Streptococcus pyogenes*.

DISCUSSION

Proper identification of *P. anaerobius* requires the use of gas chromatography; however, many hospitals do not have this type of apparatus. The technique we describe in this paper may provide an alternative approach for the separation of this organism from other anaero-

bic cocci. The zones of inhibition of *P. anaerobius* around SPS disks were 12 to 14 mm and very distinct in contrast to the indefinite zones seen with some cultures of *P. magnus* and *P. prevotii*. The latter two organisms had zones

which resembled the effect of optochin disks on some alpha hemolytic streptococci.

Schemes for the separation of anaerobic cocci without a gas chromatograph often place importance on catalase production (2); however, this type of scheme does not agree well with the V.P.I. data (6). Since many anaerobic cocci are weakly saccharolytic or asaccharolytic, the use of gas chromatography is needed for the identification of these organisms by the V.P.I. method (6). *P. anaerobius*, a weakly saccharolytic organism, will be presumptively identified with the SPS disk without the use of gas chromatography. *P. anaerobius* is the most frequently isolated anaerobic coccus at the University of Chicago Hospitals and Clinics. During the last six months, 38 of 112 anaerobic cocci isolates were *P. anaerobius*. The sources of this organism were primarily wounds, abscesses, and cervical cultures.

The mode of action of SPS on anaerobic cocci is unknown. Other data concerning inhibition of living cells by SPS deals with mycoplasma (3, 4), phagocytes (1), *Streptobacillus moniliformis* (7), and *Neisseria meningitidis* (8).

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TABLE 1. Sensitivity of various cocci to SPS

| No. of cultures | Organism | SPS ^a broth | SPS ^b disk-zone diam. (mm) |
|-----------------|------------------------------|------------------------|---------------------------------------|
| 4 | <i>P. anaerobius</i> | - | 12 |
| 11 | <i>P. anaerobius</i> | - | 13 |
| 12 | <i>P. anaerobius</i> | - | 14 |
| 11 | <i>P. asaccharolyticus</i> | + | 6 |
| 1 | ' <i>Gaffkya anaerobia</i> ' | + | 6 |
| 17 | <i>P. magnus</i> | + ^c | 6 |
| 5 | <i>P. micros</i> | + | 6 |
| 9 | <i>P. prevotii</i> | + ^c | 6 |
| 1 | <i>P. morbillorum</i> | + | 6 |
| 1 | <i>P. saccharolyticus</i> | + | 6 |
| 8 | <i>S. pneumoniae</i> | + | 6 |
| 8 | <i>S. pyogenes</i> | + | 6 |

^a SPS final concentration was 0.05% (wt/vol) in prerduced brain heart infusion broth or thioglycolate broth. Growth is represented by + and no growth by -.

^b Each 1/4" disk contained 20 μ liters of 5% SPS. Zone diameter of 6 mm indicates no inhibition.

^c Growth sometimes delayed.

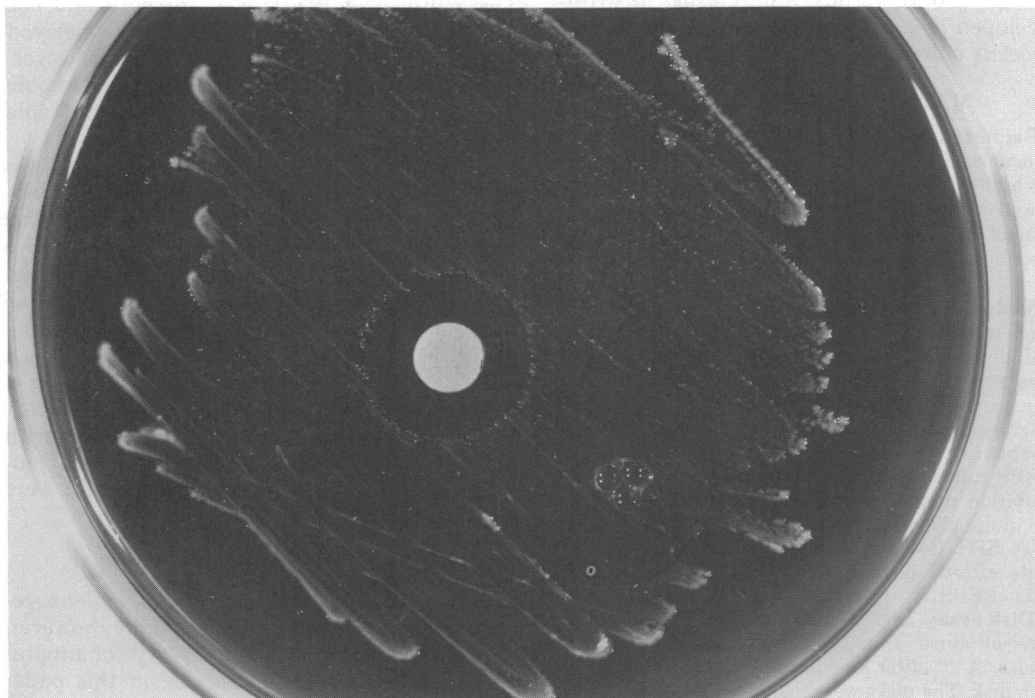


FIG. 1. Disk containing 20 μ liters of SPS on a lawn of *P. anaerobius*.

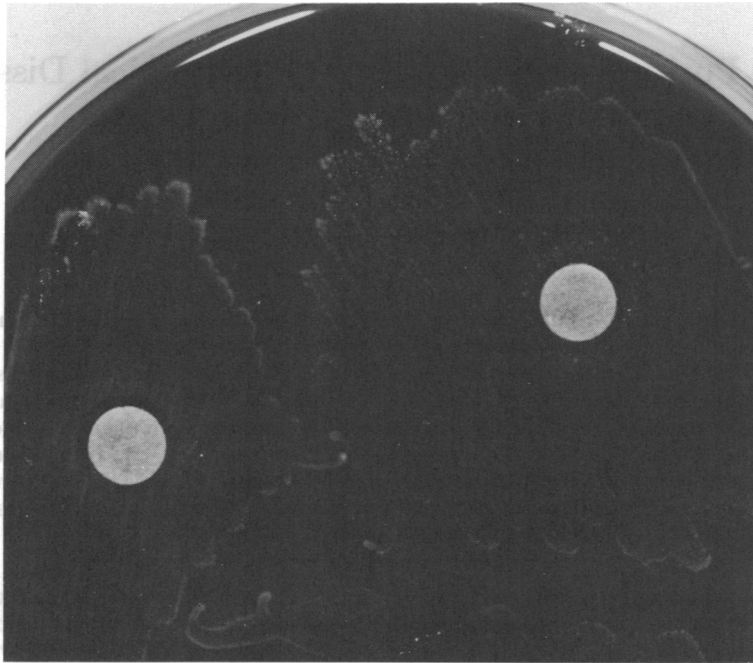


FIG. 2. Disk containing 20 μ liters of SPS on a lawn of *P. prevotii* (left) and *P. asaccharolyticus* (right).

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