Tyrosine Degradation in Presumptive Identification of *Peptostreptococcus anaerobius*

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A new tyrosine medium was developed and evaluated for the differentiation of *Peptostreptococcus anaerobius* from other anaerobic, gram-positive cocci. The strains included 159 originating from clinical specimens and 13 reference strains received from other workers in the field. Only one strain of each species was included in the study from multiple cultures from the same patient. This medium is simple to prepare and can be used in a small clinical laboratory. One hundred seventy-two strains of anaerobic gram-positive cocci were grown and evaluated with the new tyrosine medium; 36 strains (100%) of *P. anaerobius* degraded the tyrosine crystals when incubated at 37°C under anaerobic conditions in a GasPak jar (Baltimore Biological Laboratory) for approximately 72 h. On the other hand, 135 of 136 other anaerobic gram-positive cocci were negative for tyrosine degradation, but grew on the tyrosine agar plate when incubated anaerobically. The single strain that degraded tyrosine was 1 of the 13 isolates of *Peptostreptococcus micros* studied.

It is estimated today that, among cases of serious infection, anaerobes are associated with 70 to 90% of intra-abdominal infections (3), 80 to 90% of female pelvic infections (3, 13), 70 to 90% of pulmonary infections caused by aspiration (1, 3), and 90% of brain abscesses (3). Gorbach et al. (3) found *Bacterioides fragilis* in 65%, *Clostridium* in 60%, and anaerobic cocci (particularly *Peptostreptococcus* and *Peptococcus*) in 32% of abdominal infections. Holland et al. (8) found *Peptostreptococcus anaerobius* to be one of the top 10 species of anaerobes isolated in 153 specimens from 90 patients.

Recently, investigators have reported several methods to simplify the identification of clinically important anaerobic gram-positive cocci. Graves et al. (6) and Wideman et al. (16) reported that inhibition of growth of *P. anaerobius* by sodium polyanethol sulfonate could be useful for presumptive identification. Wells et al. (15) used gas-liquid chromatography to analyze the long-chain fatty acids as a tool in clarifying the taxonomy of the *Peptococcus* and *Peptostreptococcus*. Although Porschen et al. (11) and Graham et al. (5) used various serological techniques to differentiate various gram-positive anaerobic cocci with species-specific antigens, further studies are necessary to evaluate their usefulness in the clinical laboratory.

Identification of anaerobic gram-positive cocci presently includes the use of gas chromatography and biochemical tests. Many clinical laboratories do not have such equipment or identi-

fication tools available. There is a definite need for a simple, alternate identification system for the anaerobic gram-positive cocci which are isolated and associated with clinical anaerobic infections (9, 10).

A tyrosine medium was described in 1955 by Gordon and Smith (4) for use in identification of anaerobic actinomycetes. Recently the medium has been found to be of value in differentiating the *Proteus-Providencia* group and *Citrobacter diversus* from the other members of the family *Enterobacteriaceae* (12). Tyrosine degradation was also found to be useful in the identification of nonfermentative, gram-negative bacilli (V. P. Kurup and J. B. Babcock, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, C45, p. 33). Because the tyrosine medium of Gordon and Smith (4) did not adequately support the growth of certain fastidious anaerobic organisms, another medium was formulated to promote optimal growth of such organisms, and this medium was used to evaluate the value of tyrosine degradation in the presumptive identification of *P. anaerobius*. (This paper was presented in part at the XII International Congress of Microbiology, Munich, Germany, 3-8 September 1978.)

MATERIALS AND METHODS

Strains. One hundred seventy-two strains of anaerobic gram-positive cocci isolated from clinical specimens or received from other sources were studied for their ability to degrade tyrosine. Of them, 159 strains were isolated from 140 patients at the Veterans Ad-

ministration Medical Center, Wood, Wis. The remaining strains were received from the American Type Culture Collection, Rockville, Md.; Center for Disease Control, Atlanta, Ga.; Virginia Polytechnic Institute and State University, Blacksburg, Va.; and Wadsworth Veterans Administration Medical Center, Los Angeles, Calif. and are listed in Table 1. Thirteen were anaerobic, gram-positive cocci, and four were gram negative. Clinical sites from which the organisms were isolated included abscesses, soft tissues, blood, and other body fluids. Only one strain of each species was included in the study from multiple cultures from the same patient. Fresh subcultures of the strains were used in testing tyrosine degradation. During the testing period, strains were maintained either in prereduced anaerobically sterilized (PRAS) peptone yeast extract (PY) (Scott Laboratories, Inc., Fiskville, R.I.) or fluid Thioglycollate medium 135°C (Baltimore Biological Laboratory, Cockeysville, Md.) supplemented with hemin (5 µg/ml) (Difco, Detroit, Mich.), vitamin K₁ (0.1 µg/ml) (Nutritional Biochemicals, Cleveland, Ohio), and rabbit serum 10% (vol/vol). Purity of the cultures was ascertained, and the characteristic colonial morphology in different media and gram reaction of each strain were checked before testing. Representative strains of each genus and species and reference strains were lyophilized by the procedure listed in *Laboratory Methods in Anaerobic Bacteriology: CDC Laboratory Manual* (2). Lyophilized cultures were stored at ambient temperature.

Biochemical testing and gas-liquid chromatography analysis. Various procedures, described by Holdeman et al. (7), were used for the identification of clinical isolates. The results were compared with those obtained with the reference strains. For the fermentation and biochemical tests, appropriate PRAS media (Scott) were inoculated under a gentle stream of anaerobic-grade CO₂. After incubation at 37°C for 2 to 5 days, the drop in pH was measured with a combination electrode and pH meter (Beckman Instruments, Inc., Silver Spring, Md.). Isolates were tested for fermentation of cellobiose, glucose, lactose, maltose, mannose, sorbitol, sucrose, and xylose with a basal medium of PRAS-PY. The pH change in each substrate was compared with the pH of PRAS-PY and interpreted. All strains were also tested for growth in 20% bile, hydrolysis of esculin and starch, production of catalase, coagulase and indole, reduction of nitrate, and growth in a candle jar. Gram-stained smears of all strains were examined under the microscope for their morphology and staining reaction. When necessary, conversion of threonine to propionate was studied by gas-liquid chromatography analysis. For gas chromatograph analysis of acid metabolic products, the organisms were grown in PRAS-PY glucose (Scott) and incubated at 37°C for approximately 48 h. Acidification of the cultures, extraction of the volatile and nonvolatile products, and gas chromatographic procedures were followed as described by Holdeman et al. (7). The types and amounts of acids produced by various organisms were determined by comparing their retention times and peak heights with a standard concentration of known acids under the same experimental conditions. Results of gas chromatographic analysis and biochemical testing were compared with

TABLE 1. Reference strains of anaerobic cocci tested for tyrosine degradation

Organism	No. of strains	Strain no. ^a
<i>Peptostreptococcus anaerobius</i>	2	CDC 4329 WAL 4083
<i>Peptostreptococcus micros</i>	1	CDC 19367
<i>Peptostreptococcus intermedius</i>	1	WAL 4238
<i>Peptococcus magnus</i>	2	VPI 5026B WAL 2508
<i>Peptococcus asaccharolyticus</i>	1	CDC 18954
<i>Peptococcus prevotii</i>	1	VPI 5667
<i>Peptococcus indolicus</i>	1	VPI 11002
<i>Streptococcus constellatus</i>	2	CDC 18448 VPI 10378
<i>Streptococcus morbillorum</i>	2	CDC 18451 VPI 10118
<i>Veillonella parvula</i>	3	CDC 17162 VPI 8588 ATCC 10790
<i>Veillonella alcalescens</i>	1	ATCC 17745

^a CDC, Center for Disease Control, Atlanta, Ga. (V. R. Dowell, Jr.); WAL, Wadsworth Veterans Administration Medical Center, Los Angeles, Calif. (V. L. Sutter); VPI, Virginia Polytechnic Institute and State University, Blacksburg, Va. (L. V. Holdeman and W. E. C. Moore); ATCC, American Type Culture Collection, Rockville, Md.

identification tables in the *VPI Anaerobe Laboratory Manual* (7).

Medium. The new tyrosine medium consisted of the following ingredients: 44 g of Schaedler agar (Baltimore Biological Laboratory), 4 g of yeast extract (Difco), 3 g of 1-tyrosine (Nutritional Biochemicals: ICN), and 1 liter of distilled water. After adjusting the pH to 7.0, the medium was autoclaved for 15 min at 121°C. It was allowed to cool to approximately 50°C before adding vitamin K₁ solution (Nutritional Biochemicals: ICN). Vitamin K₁ solution was prepared by the methods described previously (14), filter sterilized, stored under refrigeration, and added to the medium to give a final concentration of 10 µg/ml. Approximately 20 ml of medium was poured into sterile plastic petri dishes (15 by 100 mm). Proper care was taken to disperse the tyrosine crystals uniformly throughout the medium. For the purpose of storage, the plates were refrigerated in plastic sleeves to prevent drying of the agar and were warmed to room temperature before inoculation. The medium was not reduced before inoculation. This step was avoided because it may not be feasible for a small laboratory to reduce medium. The plates were heavily inoculated with colonies from a 48-h anaerobic culture grown from an enriched blood agar plate. The inoculum was spread over a 15-mm-diameter surface of the modified tyrosine agar plate and stabbed once into the medium. Four strains can be conveniently inoculated on each plate. The plates were incubated for 1 week under anaerobic conditions with a GasPak jar (Baltimore Biological

Laboratory). Disappearance of the crystals from around and/or beneath the colony was considered a positive degradation of tyrosine (Fig. 1). No dark brown pigment was seen. A negative test was indicated by growth on the medium, with no disappearance of the crystals or brown pigment.

RESULTS AND DISCUSSION

The results of tyrosine degradation by 159 clinical strains of anaerobic, gram-positive cocci and 13 reference strains are illustrated in Table 2. All strains tested grew well in the medium, and all 36 strains of *P. anaerobius* and one *Peptostreptococcus micros* strain were positive for tyrosine degradation. The remaining 135 anaerobic gram-positive cocci were tyrosine negative.

The original tyrosine medium of Gordon and Smith (4) was described for differentiation of *Nocardia* species and therefore consisted only of a simple basal medium of peptone and beef extract. For optimal growth of anaerobic organisms, the tyrosine medium was modified by using Schaedler agar, vitamin K₁, and yeast extract. The amount of L-tyrosine was reduced from 0.5 to 0.3% (wt/vol) to make a less-dense suspension of tyrosine crystals so that the degradation could be rapidly detected. All strains tested in this study were incubated for 1 week in an attempt to detect any weak positive reactions that might develop by prolonged incubation, but none was found. However, it was found by re-inoculating the strains of *P. anaerobius* that the positive reaction could be read in 48 to 72 h after incubation if an adequate inoculum from an

TABLE 2. Results of tyrosine degradation by anaerobic gram-positive cocci

Organism	Total strains	Tyrosine degradation	
		Positive	Negative
<i>P. anaerobius</i>	36	36	0
<i>P. micros</i>	13	1	12
<i>Peptostreptococcus</i> species	7	0	7
<i>P. intermedius</i>	1	0	1
<i>P. magnus</i>	41	0	41
<i>P. asaccharolyticus</i>	28	0	28
<i>P. prevotii</i>	15	0	15
<i>P. indolicus</i>	1	0	1
<i>Peptococcus</i> species	15	0	15
<i>Ruminococcus</i> species	2	0	2
<i>Gaffkya anaerobia</i>	1	0	1
<i>Streptococcus intermedius</i>	7	0	7
<i>S. constellatus</i>	3	0	3
<i>S. morbillorum</i>	2	0	2

actively growing culture was used. It should be noted that the thickness of the agar will have some effect on the rate of the reaction and the zone size of decomposition of the crystals; generally a thicker plate will require longer incubation. Ideally, the plate should be approximately 5 mm thick. To insure proper interpretation of the reaction, if the plate has been incubated for 48 h, observation should include checking beneath the colony for degradation of the crystals beside looking for a clear zone of degradation (Fig. 1).

The single clinical strain of *Peptostreptococcus micros*, which showed tyrosine degradation early in the study, was lost on subculture. Because this study dealt with only a limited number of *P. micros* strains, more strains need to be tested before any conclusions can be made. Because *P. anaerobius* strains were frequent clinical isolates, it is felt that the tyrosine degradation offers a simple diagnostic tool to delineate *P. anaerobius* from other anaerobic gram-positive cocci in laboratories which do not have sophisticated equipment or techniques.

Eleven strains of *Veillonella*, an anaerobic gram-negative coccus that is infrequently isolated from patient specimens, were also tested for their ability to degrade tyrosine. Occasionally gram-positive cocci show various degrees of gram-negative staining and may be confused with *Veillonella*. In the present study, none of the 11 strains tested degraded tyrosine.

The new tyrosine medium is simple to prepare and therefore suitable for use in a small laboratory. Also, the medium provides a simple means to presumptively identify *P. anaerobius* and separate this organism from other anaerobic

FIG. 1. Two positive tyrosine reactions (*P. anaerobius*) indicated by a clear zone around the colony. Two negative reactions (*P. asaccharolyticus* and *P. magnus*) showing growth but no clear zone.

gram-positive cocci in laboratories which use GasPak jars or other simple equipment for providing anaerobic conditions.

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