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Extractable Antigen Shared by *Peptostreptococcus anaerobius* Strains

MITCHELL B. GRAHAM† AND WILLIAM A. FALKLER, JR.*
Department of Microbiology, University of Maryland Dental School, Baltimore, Maryland 21201

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Extracts from several species of gram-positive cocci were prepared by a modification of the Rantz-Randall autoclave method and tested for reactions with rabbit anti-*Peptostreptococcus anaerobius* (ATCC 27337 and VPI 5737) sera in a capillary precipitin test. Antigen preparations from two reference strains of *P. anaerobius* (ATCC 27337 and VPI 5737) and six clinical isolates of *P. anaerobius* reacted with the *P. anaerobius* antisera. These extracts formed a line of identity by immunodiffusion and displayed at least one precipitin line by immunoelectrophoresis. Absorption of the antisera with either the autoclaved extract or a 10% whole-cell suspension from each of the eight *P. anaerobius* strains removed the precipitin line(s) observed during immunodiffusion and immunoelectrophoresis. Extracts prepared to other species of *Peptococcus*, *Peptostreptococcus*, and *Streptococcus* did not react with the *P. anaerobius* antisera in a capillary precipitin test. In addition antisera to Lancefield groups A to G did not react with the extracts from the eight *P. anaerobius* strains. Preliminary chemical analysis of the extracts from the eight strains showed that they contained approximately 0.2 mg of carbohydrate per ml and 3.6 mg of protein per ml. The rabbit anti-*P. anaerobius* sera used in this study detected a common antigen(s) shared by strains of *P. anaerobius*, but did not react with autoclave extracts prepared from other species of gram-positive cocci. This extractable antigen could be used in a capillary precipitin test to rapidly identify *P. anaerobius* strains isolated in the clinical microbiology laboratory.

Serological techniques have been used to group and identify the streptococci beginning with the studies of Rebecca Lancefield (6). She prepared extracts from streptococcal cells and reacted them with rabbit antisera that had been prepared to streptococci isolated from animals and humans. From her studies she was able to group different species of *Streptococcus* into distinct serological groups in which members within the same group shared a common carbohydrate antigen known as the group-specific carbohydrate. Today many clinical microbiology laboratories use antisera prepared to the Lancefield groups to trace the origin of human streptococcal infections.

The peptostreptococci, which are often referred to as the anaerobic streptococci, differ from the streptococci in one very important characteristic. Unlike the streptococci, they do not produce lactic acid homofermentatively. However, there have been no reports in the literature to determine whether the peptostreptococci have the characteristic streptococcal antigens. Our previous investigation on the immunogenic reactivity of the members of the genus *Peptostreptococcus* had shown that antigens were shared between the two strains of *Peptostreptococcus anaerobius* tested (3). Because the streptococci are grouped into serological groups depending on whether they share the same group-specific carbohydrate antigen, experiments were performed to determine whether *P. anaerobius* strains had a species-specific antigen. This study reports on the isolation of an extractable antigen (EA) which is shared by *P. anaerobius* strains.

MATERIALS AND METHODS

Bacterial strains and media. The strains listed in Table 1 were used in this study. *Streptococcus mutans* (serotypes *a* to *e*), *Streptococcus salivarius* (ATCC 8618 and 13419), and *Streptococcus sanguis* (JC 43, 67, 74, and Blackburn) were obtained from H. M. Stiles, National Institute of Dental Research. Reference strains of peptococci and peptostreptococci were obtained from the American Type Culture Collection (*Peptococcus niger* ATCC 27331. *P. anaero-*

† Present address: Department of Microbiology and The Institute of Dental Research, University of Alabama in Birmingham, Birmingham, AL 35294.

bius ATCC 27337, *Peptostreptococcus productus* ATCC 27340, and *Streptococcus morbillorum* ATCC 27527) and the Virginia Polytechnic Institute (*P. anaerobius* VPI 5737, *Peptostreptococcus micros* VPI 2618, and *Peptostreptococcus parvulus* VPI 5229). Clinical isolates of peptococci and peptostreptococci were obtained from the Veterans Administration and Maryland General Hospitals located in Baltimore, Md., and were identified by the VPI *Anaerobe Laboratory Manual*, third edition (5). The streptococci were grown in Trypticase soy broth (Baltimore Biological Laboratory) and were incubated aerobically at 37°C. Overnight broth cultures were centrifuged at 10,000 × *g* for 20 min and washed three times in 0.15 M NaCl (saline). The peptococci and the peptostreptococci were grown in Schaedler broth (Baltimore Biological Laboratory) and were incubated in Baltimore Biological Laboratory anaerobe jars with GasPaks at 37°C.

Soluble antigen preparation. Soluble antigen preparations of *P. anaerobius* ATCC 27337 and VPI 5737 were prepared as previously described (3). The protein concentration of each soluble antigen preparation was determined by the method of Lowry et al. (7) with bovine serum albumin fraction V (Fisher Scientific) as a standard. Soluble antigen preparations from the two strains of *P. anaerobius* were used to prepare rabbit antisera.

Rabbit antisera. Rabbit antisera were prepared to *P. anaerobius* ATCC 27337 and VPI 5737 as previously reported (3).

In some experiments rabbit anti-*P. anaerobius* (ATCC and VPI) sera were absorbed with either the EA preparation or a 10% whole-cell suspension of each *P. anaerobius* strain. Absorbed sera were prepared by mixing equal volumes (0.1 ml) of antiserum with EA or a 10% whole-cell suspension of a *P. anaerobius* strain. Each mixture was incubated for 30 min at 37°C followed by incubation overnight at 4°C. The next day, the sera were centrifuged at 800 × *g* for 10 min to sediment antigen-antibody complexes. The absorbed sera were reacted with EA from the eight *P. anaerobius* strains by immunodiffusion.

EA preparation. EA were prepared by a modification of the Rantz-Randall autoclave method (2). A 20% suspension (packed-cell volume/total volume) of each strain in distilled water as well as 2.5, 5.0, and 10% suspensions of the *P. anaerobius* strains were autoclaved for 15 min at 121°C. After autoclaving, each extract was centrifuged for 30 min at 800 × *g*. The extracts were reacted with rabbit anti-*P. anaerobius* (ATCC and VPI) sera in a capillary precipitin test. The total carbohydrate and protein of the EA from the eight *P. anaerobius* strains were determined by the methods of Scott and Melvin with glucose as a standard (10) and Lowry et al. (7), respectively.

Immunodiffusion and immunoelectrophoresis. Double diffusion in two dimensions was performed on glass slides with 1.0% agarose (Calbiochem) in 0.05 M borate-buffered saline, pH 9.0. Glass slides overlaid with 3.0 ml of the 1.0% buffered agar gel were used for immunoelectrophoresis. The slides containing soluble antigen preparation and EA were subjected to electrophoresis in Veronal buffer, pH 8.6 (8).

RESULTS

Autoclaved extracts were prepared from 2.5, 5.0, 10.0, and 20% suspensions of *P. anaerobius* (ATCC 27337 and VPI 5737) and from the six clinical isolates of *P. anaerobius*. Each extract reacted with the anti-*P. anaerobius* (ATCC and VPI) sera in a capillary precipitin test. The extracts from the eight *P. anaerobius* strains formed a line of identity by immunodiffusion (Fig. 1) and displayed at least one precipitin line by immunoelectrophoresis. *P. anaerobius* antisera absorbed with EA or a 10% whole-cell suspension from each of the eight *P. anaerobius* strains were reacted in an immunodiffusion test against EA from the eight *P. anaerobius* strains. The absorbed sera did not form a precipitin line with any of the EA. Preliminary chemical analysis of the EA showed that they contained approximately 0.2 mg of carbohydrate per ml and approximately 3.6 mg of protein per ml.

To determine the specificity of the antigen(s), the extracts from eight *P. anaerobius* strains were reacted against antisera to Lancefield groups A to G in a capillary precipitin test. None of the extracts reacted with the Lancefield group antisera. In addition, extracts were prepared from the strains of peptococci, peptostreptococci, and streptococci listed in Table 1 and reacted against the *P. anaerobius* antisera (ATCC and VPI). None of these extracts reacted

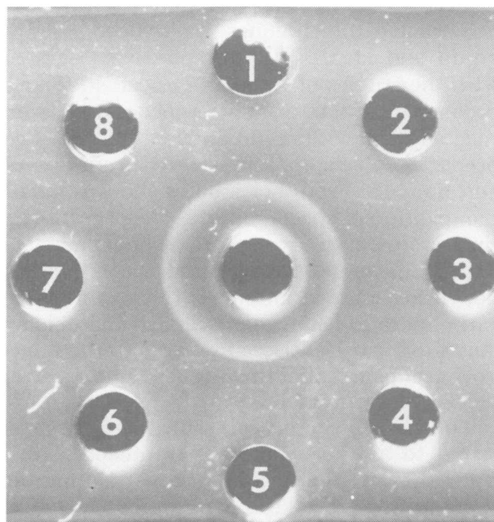

FIG. 1. *Immunodiffusion of EA from strains of P. anaerobius and rabbit anti-P. anaerobius (ATCC) serum. Center well: rabbit anti-P. anaerobius serum. Peripheral wells, P. anaerobius EA: (1) ATCC 27337; (2) VPI 5737; (3) MG-1977-1; (4) MG-6994-1; (5) VA 92-1; (6) VA B2-531-1; (7) VA B2-531-2; (8) VA 681-1.*

TABLE 1. *Bacterial strains from which autoclaved extracts were prepared*

Species	No. of strains	Sources (no.)
Microaerophilic cocci[a]	4	Soft tissue infection (3) Osteomyelitis (1)
P. asaccharolyticus	5	Soft tissue infection (4) Bacteremia (1)
P. magnus	2	Soft tissue infection (2)
P. niger	1	Umbilicus
P. prevotii	1	Soft tissue infection
Peptococcus species	3	Soft tissue infection (3)
P. anaerobius	8	Soft tissue infection (5) Bacteremia (2) Sputum
P. micros	11	Soft tissue infection (9) Subgingival crevice (1) Gastric aspirate (1)
P. parvulus	1	Facial actinomycosis (1)
P. productus	1	Bacteremia (1)
Sarcina species	1	Soft tissue infection (1)
S. morbillorum	1	Sputum
S. mutans	5	Carious lesions (5)
S. salivarius	2	Unknown (2)
S. sanguis	4	Dental plaque (4)

[a] Isolate produced a major lactic acid peak as determined by gas-liquid chromatography.

with the rabbit anti-*P. anaerobius* sera by capillary precipitin testing.

DISCUSSION

Many clinical microbiology laboratories do not attempt to identify the gram-positive anaerobic cocci to the species level for several reasons. One reason is because the clinician only needs to know that a gram-positive anaerobic coccus has been isolated from an infection to successfully treat it. Another reason is because of the tremendous cost involved in identifying them to the species level. If one were to identify the members of the genera *Peptococcus* and *Peptostreptococcus* by using either the *CDC Laboratory Manual* (1), the VPI *Anaerobe Laboratory Manual* (5), or the *Wadsworth Anaerobic Bacteriology Manual* (11), it would be necessary to inoculate several different biochemical media as well as to analyze their nonvolatile and volatile fatty acids by gas-liquid chromatography.

Several methods have been used to identify the gram-positive anaerobic cocci, such as sensitivity to antibiotics and an anticoagulant, analysis of long-chain fatty acids by gas-liquid chromatography, and indirect fluorescent antibody. Wren et al. (14) were able to separate members of the genus *Peptostreptococcus* from the genus *Peptococcus* because the peptostreptococci were sensitive to the antibiotic novobiocin, whereas the peptococci were not. In addition, Graves et al. (4) and Wideman et al. (13) reported that they were able to identify strains of *P. anaerobius* from other species of anaerobic cocci because the *P. anaerobius* strains were sensitive to sodium polyanethol sulfonate. Wideman et al. (13) reported that they found a few strains of *P. magnus*, *P. micros*, and *P. prevotii* which were sensitive to this anticoagulant. Wells and Field (12) analyzed the long-chain fatty acids of members of the genera *Peptococcus* and *Peptostreptococcus* by gas-liquid chromatography. They were unable to separate the peptococci from the peptostreptococci on the basis of their long-chain fatty acids, but they were able to identify strains of *P. anaerobius* by this method. Porschen and Spaulding (9) immunized rabbits with known strains of peptococci and peptostreptococci and reacted these antisera against known clinical strains of peptococci and peptostreptococci by an indirect fluorescent antibody test. Their antisera showed a high degree of strain specificity except the *P. magnus* antisera, which did react with other strains of *P. magnus*.

This paper reports on the isolation of a cell wall-associated antigen which was shared by the *P. anaerobius* strains tested. This EA was not shared by the other species of *Peptococcus*, *Peptostreptococcus*, and *Streptococcus* tested. Although sensitivity to sodium polyanethol sulfonate and analysis of long-chain fatty acids are used to identify *P. anaerobius* strains, we suggest that a capillary precipitin test with antisera specific for *P. anaerobius* will also allow identification of this species from other species of gram-positive anaerobic cocci.

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