Taxonomic Studies of Two Species of Peptococci and Inhibition of *Peptostreptococcus anaerobius* by Sodium Polyanethol Sulfonate

by

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TABLE OF CONTENTS

Taxonomic Studies of Two Species of Peptococci and Inhibition of *Peptostreptococcus anaerobius* by Sodium Polyanethol Sulfonate

INTRODUCTION

Anaerobic cocci have been implicated in human disease since the 1890's. They have been isolated from infections ranging in severity from simple skin infections to subacute bacterial endocarditis and septicemia. The range of pathological conditions in which anaerobic cocci have been involved includes puerperal sepsis, gingivitis, sinusitis, appendicitis, peritonitis, osteomyelitis, gangrene, and various types of abscesses. Approximately 23 to 30 percent of all anerobic isolates from pathological material are anaerobic cocci (49,50,70).

Early investigators attempted to identify and name these anaerobic cocci, but unfortunately the techniques necessary to distinguish adequately one group of anaerobic cocci from another were not then available. Therefore, several rather sketchy descriptions of the same organism were sometimes published by different persons, and different names were frequently applied to the same group of organisms. A review of the research on the Gram-positive anaerobic cocci from 1893 to 1947 and the taxonomic relationships among these organisms can be found in the doctoral dissertation of E.L. Foubert (15).

Even today, very few microbiologists in clinical laboratories identify their isolates of anaerobic cocci by species. Rather, they usually report the percentage of "anaerobic cocci," "anaerobic strep," or, at best, "*Peptococcus*" or "*Peptostreptococcus*," in a specific pathological condition. As additional knowledge of the anaerobic cocci has become

available, though, a few investigators have realized that accurate identification of these species may help elucidate the role of anaerobic cocci in disease. For example, Lambe et al. (39), in studying the role of anaerobic cocci in human disease, reported that Peptococcus asaccharolyticus, Peptococcus magnus, Peptococcus prevotii, Peptococcus variabilis, Peptostreptococcus anaerobius, Veillonella alcalescens, and Veillonella parvula were the most commonly isolated species of anaerobic cocci. All of these organisms were isolated from clinical material as pure cultures. Werner and Rintelen (77) studied the incidence of Peptococcus species isolated from pathological material and found that P. variabilis, P. asaccharolyticus, and P. prevotii were isolated frequently, often in pure culture. Porschen and Spaulding (50) found the following distribution of anaerobic cocci over a three-year period: P. prevotii, 6%; Peptostreptococcus anaerobius, 4.8%; Streptococcus intermedius, 4.1%; P. asaccharolyticus, 3.9%; and P. magnus, 3.6%. Two laboratories (1,18) have reported that Peptostreptococcus anaerobius was the anaerobic coccus most frequently isolated from clinical specimens. Holland et al. (31), in a fifteen-year study of anaerobic bacteria isolated from clinical specimens, found that P. magnus was the species of anaerobic cocci most frequently isolated and that P. asaccharolyticus, P. prevotii, Peptostreptococcus anaerobius, and S. intermedius were among the ten species of anaerobes most frequently isolated in their study. Obviously, then these species of anaerobic cocci have pathogenic potential. It is interesting that these pathogenic species belong to only four of twelve recognized genera of anaerobic cocci, and that the majority of these pathogenic species belong to the two genera Peptococcus and Peptostreptococcus.

In studying the role of microorganisms in disease, one needs to know the natural habitat of the organisms. The pathogenic anaerobic cocci have all been isolated from various body sites of healthy individuals: e.g. skin, mouth, upper respiratory tract, intestinal tract, and urogenital tract. Table 1 lists those species of anaerobic cocci which have been isolated from these areas of the human body. Table 1 also includes those other species of anaerobic cocci which are infrequently or never isolated from infections, but which nevertheless are part of the normal flora of the human body. These non-pathogenic species are mainly found in the intestinal tract.

Table 1. Anaerobic cocci found in the normal flora of the human body.

Source Species	Source Species
Mouth (65)	Vagina (65)
Megasphaera elsdenii	"*Gaffkya*" *anaerobia*
Peptococcus magnus	*Megasphaera elsdenii*
Peptococcus prevotii	*Peptococcus asaccharolyticus*
Peptostreptococcus anaerobius	*Peptococcus magnus*
Peptostreptococcus micros	*Peptostreptococcus anaerobius*
Streptococcus constellatus	*Streptococcus constellatus*
Streptococcus intermedius	*Streptococcus intermedius*
Streptococcus morbillorum	
Veillonella alcalescens	Stomach, Small Intestine (47)
Veillonella parvula	*Veillonella alcalescens*
	Veillonella parvula
Nose, Ear, Throat (65)	
Peptococcus asaccharolyticus	Large Intestine (30, 46)
Peptostreptococcus micros	*Acidaminococcus fermentans*
Streptococcus constellatus	*Gemminger formicilis*
Streptococcus intermedius	*Coprococcus catus*
Streptococcus morbillorum	*Coprococcus comes*
Veillonella alcalescens	*Coprococcus eutactus*
Veillonella parvula	*Peptococcus asaccharolyticus*
	Peptococcus magnus
Eye (44)	*Peptostreptococcus productus*
Peptococcus anaerobius	*Ruminococcus albus*
	Ruminococcus bromii
Skin (65)	*Ruminococcus callidus*
Peptococcus saccharolyticus	*Ruminococcus flavefaciens*
Peptococcus niger[a]	*Ruminococcus gnavus*
	Ruminococcus lactaris
Urine (65)	*Ruminococcus obeum*
Peptococcus prevotii	*Ruminococcus torques*
Peptococcus variabilis	*Streptococcus constellatus*
Streptococcus intermedius	*Streptococcus hansenii*
Veillonella alcalescens	*Streptococcus intermedius*
Veillonella parvula	*Streptococcus morbillorum*

a. As reported in this thesis.

Classification of the Anaerobic Cocci

HISTORICAL BACKGROUND

The taxonomy of the anaerobic cocci has been changing constantly since 1933 when Prévot (51) summarized and emended the descriptions of the species of anaerobic cocci which had previously been isolated and described. Prévot placed the anaerobic cocci in the genera Streptococcus, Diplococcus, Staphylococcus, Micrococcus, Gaffkya, Sarcina, Neisseria, and Veillonella. Gram reaction and cellular morphology were the criteria used to separate these genera. Physiological characteristics were used only to separate groups at the species level. In the classification scheme used by Prévot, the Gram-positive anaerobic cocci were placed in the family Micrococcaceae which was divided into three tribes: Streptococceae, Staphylococceae, and Micrococceae. The criterion for differentiating one tribe from another was the manner in which cell division occurred (in one or two axes and or planes). The tribe Streptococceae contained two genera: Diplococcus, with cells arranged singly or in pairs; and Streptococcus with cells arranged in chains. The tribe Staphylococceae contained the genera, Gaffkya, with cells in tetrads; and Staphylococcus, with cells in groups. The tribe Micrococceae contained the genera, Sarcina, with cells in packets of eight; and Micrococcus, with cells in irregular masses. Gram-negative anerobic cocci were placed in the genera Neisseria and Veillonella.

In 1936 Kluyver and van Niel (36) reorganized the classification scheme of all microorganisms. Unlike Prévot (51), these authors used physiological characteristics to group organisms at the genus level. Kluyver and van Niel (36) placed all cocci in the family Micrococcaceae

which was subdivided into three tribes: Sarcinae, Micrococceae, and Streptococceae. Both physiological and morphological characteristics were used to separate the cocci into different genera. Kluyver and van Niel created the two genera Peptococcus and Peptostreptococcus to include the Gram-positive anaerobic cocci capable of "fermenting protein decomposition products." These two apparently closely related genera were placed in separate tribes on the basis of morphological characteristics. Chain formation distinguished the genus Peptostreptococcus from the genus Peptococcus in the tribe Micrococceae.

Kluyver and van Niel (36) placed the Gram-negative anaerobic cocci characterized as "fermenting protein decomposition products" in the genus Veillonella. The methane-producing cocci were placed in the genera Methanococcus and Methanosarcina. The homo-fermentative lactic acid cocci were placed in the genus Streptococcus. The genera Neisseria, Micrococcus, Gaffkya, and Sarcina, which previously had contained both aerobic and anerobic species, contained cocci which possessed an "obligatory oxidative katabolism" (36). The anaerobic sarcina were placed in the two genera Zymosarcina and Butyrisarcina.

Many investigators did not immediately accept the genera Peptococcus and Peptostreptococcus and continued to classify the Gram-positive anaerobic cocci in Streptococcus, Staphylococcus, Micrococcus, Diplococcus, Gaffkya, and Sarcina. Prévot's classification scheme (51) was used in this country until 1957 when the 7th edition of Bergey's Manual of Determinative Bacteriology recognized the genera Peptococcus (13) and Peptostreptococcus (64). The French taxonomists continued to use Prévot's classification scheme even after 1957 and only now are begining

to accept the classification scheme published in Bergey's Manual of Determinative Bacteriology.

Foubert and Douglas (16), in 1948, studied the anaerobic members of the genera Staphylococcus and Micrococcus. These investigators described several new species (Micrococcus prevotii, M. saccharolyticus, and M. variabilis) and proposed a key for identification of the anaerobic cocci based on a few biochemical characteristics.

Hare and co-workers (23,24,25,71), in the 1950's, realized that it was difficult to identify anaerobic cocci according to the earlier schemes based primarily on morphological differences. These investigators recognized and studied the effect that media and various media components such as oleic acid and sulphur compounds had on the growth and biochemical reactions of anaerobic cocci. Hare and colleagues proposed an identification scheme which used gas formation, fermentation of carbohydrates, and pattern of sensitivities to various antibiotics as criteria for separation of similar strains into groups. These workers placed the anaerobic cocci into nine groups designated I through IX, but did not attempt to relate these groups to previously named species.

In 1971 Rogosa established two new families Peptococcaceae (56) and Veillonellaceae (55) for the anaerobic cocci. Four genera of Gram-positive anaerobic cocci, Peptostreptococcus, Peptococcus, Ruminococcus, and Sarcina (57), were placed in the family Peptococcaceae. The three genera of Gram-negative anaerobic cocci, Veillonella, Acidaminococcus, and Megasphaera, were placed in the family Veillonellaceae (55).

CURRENT CLASSIFICATION OF THE ANAEROBIC COCCI

The families, genera, and species of anaerobic cocci included in Bergey's Manual of Determinative Bacteriology, 8th ed. (6,7,9,58,59), or recognized as distinct species by Holdeman and Moore (28,29), or described as new species since 1970, are listed in Table 2. In these classification schemes, the anaerobic cocci belong to the families Peptococcaceae, Streptococcaceae, Veillonellaceae, and Methanobacteriaceae. Species are placed in these families on the basis of Gram reaction and physiological differences. The Gram-negative anaerobic cocci are placed in the family Veillonellaceae. The Gram-positive anaerobic cocci are placed in the families Peptococcaceae, Streptococcaceae, and Methanobacteriaceae.

Gram-negative Anaerobic Cocci

The family Veillonellaceae contains three genera -- Veillonella, Acidaminococcus, and Megasphaera (55). Another genus of Gram-negative anaerobic cocci, Gemminger (17), contains the single species G. formicilis. The Gram-negative anaerobic cocci are separated into species on the basis of energy sources and metabolic endproducts. Species in the family Veillonellaceae generally possess a cellular morphology of diplococci, often with adjacent sides appearing to be flattened.

The genus Veillonella (58) contains those cocci which produce propionic acid from lactate and pyruvate. Rogosa (58) has reported that these organisms do not ferment carbohydrates, and that the two species V. parvula and V. alcalescens are differentiated on the basis of hydrogen peroxide degradation by a non-heme protein. Mays and Johnson (personal

Table 2. Currently recognized classification of anaerobic cocci.[a]

Family	Genus	Species
Peptococcaceae	Peptococcus	niger[b] activus aerogenes anaerobius asaccharolyticus heliotrinreducans (41) indolicus (67,68) magnus prevotii saccharolyticus (29) variabilis
	Peptostreptococcus	anaerobius lanceolatus micros parvulus productus
	Ruminococcus	flavefaciens albus bromii (45) callidus (28) gnavus (48) lactaris (48) obeum (48) torques (28)
	Sarcina	ventriculi maxima
	Coprococcus (28)	eutactus (28) catus (28) comes (28)
Streptococcaceae	Streptococcus	constellatus (28) hansenii (28) intermedius (28) morbillorum (28)

Table 2. continued.

Family	Genus	Species
Methanobacteriaceae	Methanococcus	mazei vanielii
	Methanosarcina	methanica barkeri
Veillonellaceae	Veillonella	parvula alcalescens[c]
	Acidaminococcus	fermentans
	Megasphaera	elsdenii
unassigned	Gemminger	formicilis (17)
	"Gaffkya"[d]	anaerobia (29)

[a] Species listed here are described in Bergey's Manual of Determinative Bacteriology, 8th ed., Williams and Wilkins Co., Baltimore, or the reference for the species description is given in parentheses.

[b] An emended description is given in this thesis.

[c] Mays and Johnson (personal communication) found by DNA hybridization studies that these two species are related genetically (76% homology) and, therefore, should be considered as a single species, V. parvula. This observation will appear in the Abstr. of the Ann. Meeting, Am. Soc. for Microbiol., 1977.

[d] The genus "Gaffkya" is not a recognized taxon (33).

communication) have found that the deoxyribonucleic acid (DNA) of the neotype strains of these two species is 76% homologous and, therefore, suggest that these two species should be considered as one species, V. parvula.

Amino acids are the sole energy source for Acidaminococcus fermentans, the only species in the genus Acidaminococcus (53). Glutamic acid is the preferred substrate. Acetic and butyric acids and CO_2 are the major fermentation products. Carbohydrate fermentation, if present, is very weak.

Megasphaera elsdenii (19,54), the only species in the genus Megasphaera, utilizes glucose, fructose, and lactate. The fermentation products are formic, acetic, propionic, iso-butyric, butyric, valeric, and caproic acids, and CO_2. Caproic acid is the main endproduct from glucose fermentation, but not from lactate utilization. Megasphaera elsdenii is the largest of the anaerobic Gram-negative cocci with a cell diameter of 2.0 to 2.6 μm. When this species was originally named, it was placed in the genus Peptostreptococcus (19). However, Rogosa (54) has demonstrated by electron micrographs that this organism actually has a Gram-negative type cell wall and therefore, does not conform to the description of the genus Peptostreptococcus. This organism also does not conform to the descriptions of the two Gram-negative genera. Therefore, Rogosa created the new genus Megasphaera (54) and included it in the family Veillonellaceae (55).

Gemminger formicilis (17), isolated from the intestines of men and chickens, has a cell wall with a characteristic Gram-negative structure and divides by "budding." Because of this "budding" type of division,

G. formicilis cannot be included in the family Veillonellaceae, because this family includes only those Gram-negative spherical cocci which divide to form diplococci. Therefore, the genus Gemminger has not been assigned to a higher taxon. G. formicilis ferments carbohydrates and produces formic and butyric acids as major endproducts and, in addition, produces smaller amounts of acetic, pyruvic, lactic, succinic, and malonic acids.

Gram-positive Anaerobic Cocci

The Gram-positive anaerobic cocci are placed in the families Peptococcaceae, Streptococcaceae, and Methanobacteriaceae. Genera are placed in these families on the basis of physiological differences.

Gram-positive anaerobic cocci which produce methane are placed in either of the two genera Methanococcus (7) or Methanosarcina (6) in the family Methanobacteriaceae. These two genera are distinguished from one another on the basis of cellular morphology; the cells of Methanosarcina are characteristically arranged in packets, whereas the cells of Methanococcus are arranged singly, in pairs, or in irregular groups. To obtain energy, methanococci produce methane from H_2, CO_2, and formate; methanosarcina produce methane from H_2, CO_2, formate, and methanol. These organisms have been isolated from swamps, sewage digestor sludge, and garden soil.

Cocci which ferment carbohydrates and produce lactic acid as the major fermentation product are placed in the genus Streptococcus in the family Streptococcaceae (11). This genus contains both aerobic and anaerobic species. Two anaerobic species, S. constellatus and

S. morbillorum, both of which produce lactic acid as the major fermentation product, were transferred from the genus Peptococcus because they did not conform to the description of this genus (28). Early descriptions of these species had placed them in the genus Diplococcus in the tribe Streptococceae (51). Peptostreptococcus intermedius was returned to the genus Streptococcus (28).

The family Peptococcaceae, as established by Rogosa (56) contains species of Gram-positive anaerobic cocci which produce, as metabolic endproducts, short chain fatty acids, and sometimes also succinate, ethanol, CO_2, H_2, and H_2S. Lactic acid may be produced, but it is not a major product. The family Peptococcaceae contains five genera: Peptococcus, Peptostreptococcus, Ruminococcus, Sarcina, and Coprococcus.

The genus Ruminococcus requires a fermentable carbohydrate and frequently requires rumen fluid or fecal extracts for abundant growth (45,59). The major endproducts from carbohydrate fermentation are acetic and formic acids, and sometimes succinic acid or ethanol. Ruminococci have been isolated from human feces, bovine and ovine rumens, and from the ceca of rabbits and guinea pigs.

The genus Coprococcus (28), like the genus Ruminococcus, is composed of species which actively ferment carbohydrates; however, butyric and acetic acids are produced as the major fermentation products. Carbohydrates are either required for growth or greatly stimulate growth.

The genus Sarcina (9) contains those anaerobic cocci that divide in three perpendicular planes and therefore occur in cubical packets. The two species S. ventriculi and S. maxima both ferment carbohydrates and are capable of growing at pH values of 2.0 to 2.5. This genus

previously contained numerous aerobic and two anaerobic methanogenic species, all since transferred to other genera (8).

The two genera Peptococcus and Peptostreptococcus, which contain most of the anaerobic cocci isolated from infections, are plagued with a large number of taxonomic problems. The major taxonomic problem concerning these two genera is the striking similarity between them. Both genera are physiologically quite similar: peptones or amino acids are used by both as the main nitrogen and energy source, the guanine plus cytosine content of the DNA (% G+C) values fall in similar ranges (Table 3), carbohydrate fermentation is variable among the species of the species of both genera, short chain fatty acids containing more than three carbon atoms may be produced by species in both genera, and cellulose is not digested by species in either genus. These are all physiological characteristics which have been used to differentiate the genera in the family Peptococcaceae (59).

The major distinguishing characteristic between the two genera Peptococcus and Peptostreptococcus, as originally described by Kluyver and van Niel (36) and by Rogosa (59), was a difference in cellular morphology. Members of the genus Peptostreptococcus were described as occuring in pairs and chains, whereas members of the genus Peptococcus were described as occurring singly, in pairs, tetrads, or in irregular groups. However, this chain-forming ability of peptostreptococci cannot be used to distinguish between these two genera. Only Peptostreptococcus anaerobius and P. productus in the genus Peptostreptococcus consistently occur in chains; P. micros and P. parvulus, two other species in the genus, are found in pairs, irregular groups, and short chains of five to

six cells (29). Several species in the genus Peptococcus (P. asaccharolyticus, P. magnus, P. prevotii, P. saccharolyticus, and P. variabilis) also occurs in short chains of four to six cells in addition to pairs and irregular groups (29). Therefore, cellular morphology obviously does not appear to be a characteristic which can be used to distinguish between these genera.

Peptostreptococcus productus is a species which probably does not belong in the genus Peptostreptococcus for the following reasons: the % G+C of the DNA of P. productus is dissimilar from the % G+C of the DNA of other species in the genus Peptostreptococcus, (Table 3), the cells frequently may be elliptical to "extremely elongate and may appear as tapered rods" (46) rather than the cells being spherical as characteristic of the genus Peptostreptococcus, and this species is very saccharolytic (29, 46). Moore and Holdeman (46) have stated that "Peptostreptococcus productus strains form a continum of phenotypes." Moore (personal communication) has suggested that this group of anaerobic cocci probably represents several distinct species that do not belong in the genus Peptostreptococcus.

Additional taxonomic problems involve Peptococcus niger, the type species of the genus Peptococcus, Peptococcus anaerobius, Peptococcus prevotii, Peptococcus asaccharolyticus, Peptococcus activus, Peptococcus aerogenes, and Peptostreptococcus lanceolatus.

Strains of the type species of the genus Peptococcus, Peptococcus niger, are not extant. The description of this species was based on one strain isolated by I. C. Hall (21) in 1930. This one strain has been lost and additional strains of this organism have not been isolated.

Peptococcus anaerobius and Peptostreptococcus anaerobius are very similar in name. The same specific epithet is used for both genera, and the accepted abbreviation for both is "P". This similarity in names could be confusing, especially in clinical situations. And to increase the confusion further, several species of anaerobic cocci are considered as synonyms of Peptococcus anaerobius. These are Peptococcus magnus, Peptococcus glycinophilus, and Peptococcus variabilis (59).

The original description of Peptococcus prevotii (16) was based on two distinct organisms. One of these is the non-saccharolytic organism recognized today as P. prevotii by Holdeman and Moore (29); the other is a saccharolytic organism which corresponds to the description of "Gaffkya" anaerobia, an organism whose generic name has been rejected (33). Rogosa considers the non-saccharolytic element of P. prevotii to be indistinguishable from Peptococcus asaccharolyticus. However, Holdeman and Moore (29) differentiate P. prevotii from P. asaccharolyticus on the basis of indole production, and therefore consider these species to be separate and distinct.

The generic name "Gaffkya" was rejected by the Judicial Commission of the International Committee on Systematic Bacteriology (33,38), and so a legitimate name for this distinct group of organisms does not exist. "Gaffkya" anaerobia (29) is a Gram-positive coccus which produces butyric and lactic acids and ferments a few carbohydrates. The cellular morphology is characteristically in pairs, tetrads, or clumps.

Several species included in Bergey's Manual of Determinative Bacteriology, 8th ed. (59), are not recognized by other workers studying the anaerobic cocci (14,29,70). These species are Peptococcus activus,

Peptococcus aerogenes, and Peptostreptococcus lanceolatus, and all could be considered synonyms of recognized species of anaerobic cocci. Type strains of these species do not exist and only one strain of P. aerogenes has been deposited with any culture collection. The strain of P. aerogenes (ATCC 14963, H.C. Douglas strain UW 228 ← A.R. Prévot BAI [Staphylococcus aerogenes]), deposited with the American Type Culture Collection, Rockville, Md., has been identified as P. asaccharolyticus by Holdeman and Moore (personal communication). Rogosa (59) has suggested that P. aerogenes in regard to morphological characteristics, general cultural characteristics, amino acid metabolism, and % G+C of the DNA is indistinguishable from P. asaccharolyticus. Therefore, P. aerogenes is probably synonymous with either P. asaccharolyticus or P. prevotii, two species distinguished from each other by indole production.

These taxonomic problems, coupled with differing identification schemes (14,29,70), have made it difficult for clinical microbiolgoists to identify anaerobic cocci consistently and accurately. Holland et al. (31) clearly pointed out this difficulty. Using morphological characteristics and hydrogen peroxide decomposition as criteria for differentiating the genera Peptococcus and Peptostreptococcus (14), these authors found that 78% of their isolates of anaerobic cocci were Peptostreptococcus species. Using a different identification scheme (29), they found that only 31% of these same strains were Peptostreptococcus species species. Currently there are three widely-accepted identification schemes for anaerobic bacteria that are used in clinical laboratories. These are Laboratory Methods in Anaerobic Bacteriology, CDC Laboratory Manual (14), The Wadsworth Anaerobic Bacteriology Manual (70), and the VPI & SU Anaerobe Laboratory Manual (29).

The VPI & SU Anaerobe Laboratory Manual (29) and The Wadsworth Anaerobic Bacteriology Manual (70) recognize the same species of anaerobic cocci and use the same criteria for identification. The Laboratory Methods in Anaerobic Bacteriology, CDC Laboratory Manual (14) differs from the two above in that peptococci and peptostreptococci are are placed in "CDC groups" instead of being identified as currently accepted species. Before 1974, catalase production was a key characteristic used in distinguishing peptococci from peptostreptococci in the CDC manual (14). This can account for the results obtained by Holland et al. (31) as mentioned above. Graves et al. (18) also mention the confusion that can occur when catalase is used to identify anaerobic cocci. However, since 1974, morphological differences, energy sources, and fermentation products are used as key characteristics in distinguishing the various genera of the family Peptococcaceae in the CDC manual (additional handout, V.R. Dowell, Jr., personal communication.)

The classification of anaerobic cocci continues to change as better methods of distinguishing groups of organisms from one another are discovered or applied. Some of these recently applied methods are the determination of % G+C ratios of the DNA of microorganisms, DNA-DNA hybridization studies which indicate the genetic interrelatedness of groups, cell wall analyses, immunological studies, and comparsion of fatty acid content of groups of organisms. All of these techniques, except DNA-DNA hybridization, have been applied to the study of the taxonomy of the anaerobic cocci, especially to the genera Peptococcus and Peptostreptococcus.

Determination of the guanine plus cytosine ratios of the DNA of

microorganisms has been very helpful in grouping organisms into specific genera. For example, many of the aerobic species of Sarcina had % G+C values very different from those of the anerobic species; therefore, these aerobic species were transferred to other genera (8). The % G+C values of various anaerobic cocci are given in Table 3.

Schleifer and Nimmerman (61) determined the cell wall composition for eight strains in the genus Peptococcus. They found that at least four types of peptidoglycan occur in this group. The peptidoglycan of P. saccharolyticus was similar to the peptidoglycan of coagulase negative aerobic staphylococci and was distinctly different from the peptidoglycan types of the other three groups. The species P. prevotii and P. aerogenes had the same interpeptide structure; the peptidoglycans of P. prevotii and P. variabilis possess similar peptide subunits. These authors believe that, because of the interrelatedness of their peptidoglycans, P. prevotii, P. aerogenes, and P. variabilis should be placed in the same genus.

Bahn et al. (3) studied the chemical composition of cell walls of five strains of Peptostreptococcus. These authors also analyzed the cell walls serologically. The cell walls of the two P. putridus (Peptostreptococcus anaerobius) strains contained glucose, mannose, glutamic acid, aspartic acid, alanine, lysine, muramic acid, glucosamine, and galactosamine. The cell walls of the two P. intermedius (S. intermedius) strains were composed of the same compounds as P. putridus, except that rhamnose was substituted for mannose and very little aspartic acid was present. The P. intermedius strains reacted weakly with streptococcal grouping antisera (one strain with group C; the other strain with groups

Table 3. Guanine plus cytosine content of the deoxyribonucleic acid of various species of anaerobic cocci.

Species	% G+G	reference
Peptococcus niger	50	*
Peptococcus aerogenes	35.7-36.7	53
Peptococcus asaccharolyticus	36	59
Peptococcus heliotrinreducans	35.4-36.9	41
Peptococcus saccharolyticus	30-31	45
Peptostreptococcus anaerobius	33.7-36.2	60
Peptostreptococcus lanceolatus	30.7-33.7	60
Peptostreptococcus micros	27.9-31	60
Peptostreptococcus productus	43.2-44.8	60
Ruminococcus flavefaciens	39.8-43.5	59
Ruminococcus albus	42.6-45.4	59
Ruminococcus bromii	39-40	45
Ruminococcus callidus	43	28
Ruminococcus gnavus	43	48
Runinococcus lactaris	45	48
Ruminococcus obeum	40-41	48
Ruminococcus torques	40-42	28
Sarcina ventriculi	30.6	8
Sarcina maxima	28.6	8
Coprococcus eutactus	41	28
Coprococcus catus	39-41	28
Coprococcus comes	40-42	28
Streptococcus hansenii	37-38	28
Streptococcus intermedius	38.2-39, 38.7	28,60
Veillonella parvula	41.3-44.4	58
Veillonella alcalescens	40.3-44.4	58
Acidaminococcus fermentans	56±0.9	53
Megasphaera elsdenii	53.6±0.5	58
Gemminger formicilis	59	17

* Reported in this thesis.

E and F), whereas the other three strains did not react at all. P. elsdenii (Megasphaera elsdenii) cell walls contained aspartic acid, glutamic acid, alanine, glycine, diaminopimelic acid, teichoic acid, muramic acid, glucosamine, and galactosamine, traces of lysine, and very little carbohydrate (3.1%).

Lambe et al. (40) studied ways of differentiating S. constellatus, S. intermedius, and S. morbillorum from other streptococci. They found that only two of thirty strains reacted with streptococcal grouping antisera; one strain reacted with group C, and the other strain reacted with group F. Both strains were identified as S. intermedius. These authors did find that these species could be differentiated from other streptococci on the basis of hippurate hydrolysis, beta hemolysis of blood, bile-esculin reaction, and growth in 6.5% NaCl.

Porschen and Spaulding (50) have studied the serological relatedness of pathogenic species of Peptococcus and Peptostreptococcus with flourescent antibody techniques. They did not find a common Peptococcus or Peptostreptococcus antigen. However, P. magnus had a major species specific antigen that could be detected with the flourescent antibody technique. Flourescent antibody conjugates, prepared from P. asaccharolyticus, P. prevotii, Peptostreptococcus anaerobius, and S. intermedius, had a high degree of strain specificity but not species specificity.

The cellular long-chain fatty acids of peptococci and peptostreptococci were studied by Wells and Field (76) in an attempt to clarify the taxonomic relationships among these organisms. These authors studied twelve species (82 strains) and were able to place them into four groups on the basis of long-chain fatty acid analyses. Peptostreptococcus

anaerobius was placed in a group by itself because the chromatograms showed the largest number of fatty acid peaks and a characteristic peak between C_8 and C_{10}. P. intermedius, P. micros, P. parvulus, P. morbillorum, and P. constellatus all produced C_{14}, $C_{16:1}$, $C_{18:1}$, and C_{18} fatty acids and were placed in group II. It is of interest that these organisms are the only species of anaerobic cocci which produce lactic acid as the major fermentation product. Group III cocci contained $C_{16:1}$, C_{16}, $C_{18:1}$, and C_{18} fatty acids like group II and, in addition, contained three to six peaks which were not identified. Organisms included in group III are P. variabilis, P. magnus, P. asaccharolyticus, P. prevotii, and P. productus (1 strain). The single species P. saccharolyticus contained an unidentified peak between C_{14}, and C_{16}, C_{14}, C_{16}, $C_{18:1}$, C_{18}, and C_{20} fatty acids and so was placed in group IV. Wells and Field believe that P. saccharolyticus is significantly different from other peptococci and should be placed in a group by itself.

The studies described above have shed some light on the proper classification of anaerobic cocci. The species of anaerobic cocci which produce lactic acid show definite similarities with the facultative streptococci, and therefore these studies support the transfer of these lactic acid-producing species (S. constellatus, S. morbillorum, and S. intermedius) to the genus Streptococcus (28). The cell wall analysis of Schleifer and Nimmerman (61) and the long-chain fatty acid analysis of Wells and Field (76) both indicate that P. saccharolyticus is different from other peptococci and should not be included in the genus Peptococcus. However, neither of these authors suggested in which genus this species should be placed.

Unfortunately, these studies, with the exception of the long-chain fatty acid analysis, were all performed using a very small number of strains (five to eleven). Therefore, these studies do not take into account the possibility of strain-to-strain variation. Thus, a large number of strains should be studied before definite nomenclatural changes can be made.

STATEMENT OF THESIS PROBLEM

This thesis will examine taxonomic problems concerning two species in the genus Peptococcus and will also study the inhibition of growth of Peptostreptococcus anaerobius by sodium polyanethol sulfonate.

Peptococcus niger, an anaerobic coccus which forms black colonies on blood agar plates, is the type species of the genus Peptococcus. The original strain isolated by Ivan C. Hall is lost; the one other strain that has been isolated lost its ability to form black colonies, and therefore its identification as P. niger is questionable. The receipt of a strain of a black colony-forming anaerobic coccus, isolated from the navel of an astronaut, has stimulated new interest in this taxonomically important species and, in addition, has suggested the natural habitat of these organisms. Therefore, a survey of the flora of navels of humans will be undertaken in an attempt to isolate and characterize additional strains of Peptococccus niger.

The taxonomic status of Peptococcus anaerobius is uncertain because the original isolates are no longer extant and, in addition, the original description of this species is vague. A comparsion of the original description of Peptococcus anaerobius with descriptions of other species of anaerobic cocci which could be considered synonymous with Peptococcus anaerobius will be undertaken. In addition, strains identified by A.R. Prévot will be compared to strains of these possibly synonymous species in an attempt to elucidate the taxonomic status of Peptococcus anaerobius.

The inhibition of growth of Peptostreptococcus anaerobius by sodium polyanethol sulfonate, a compound added to blood culture media to prevent clotting of the blood and to eliminate the bactericidal action of blood,

will also be studied. J. Mehl of the Becton-Dickinson Company has suggested that the growth of Peptostreptococcus anaerobius is not inhibited in certain media. However, neither the medium component(s) that neutralize this inhibitory effect nor the mechanisms of the anti-inhibitory factor is known. These factors will be investigated. The sensitivity of other pathogenic species of anerobic cocci to sodium polyanethol sulfonate will also be studied.

Peptococcus niger (Hall) Kluyver and van Niel 1936: Emendation of Description and Designation of Neotype Strain

INTRODUCTION

In 1930, I. C. Hall isolated and described an anaerobic Gram-positive coccus that formed black-pigmented colonies (21) and named it *Micrococcus niger*. Later, when Kluyver and van Niel created the genus *Peptococcus* (36), they designated this organism the type species of the genus. Hall's description was based on a single strain isolated from the urine of an aged woman (21). The original strain is no longer extant. We have not found it in any type culture collection nor in Hall's personal collection of anaerobic bacteria, which was given to the Virginia Polytechnic Institute and State University (VPI & SU) Anaerobe Laboratory. However, we did find Hall's original records and worksheeets, which have been of considerable help in determing the characteristics of the original strain of *Peptococcus niger* (Hall) Kluyver and van Niel.

P. niger (Hall) Kluyver and van Niel was originally described by Hall (21) as an obligately anaerobic coccus about 0.6 µm in diameter forming irregular masses resembling staphylococci. This organism formed colonies (0.5mm in diameter) that were dark black, irregularly globular, smooth, and dense in 1% meat infusion agar with 2% peptone. Upon exposure to air these colonies faded to a dull gray color. On blood agar the colonies in 5 days "arose like tiny black pearls, round, smooth, and glistening." The organism was nonhemolytic, did not liquefy gelatin or serum, did not digest milk, and did not ferment carbohydrates. This organism did produce H_2S.

During Bacteriological monitoring by technologists of Brown, Root, and Northrup, Houston, Tex., of the flora of three astronauts participating in a mock Skylab flight, cocci that formed black colonies on blood agar plates were repeatedly isolated from swabs of the navel of one astronaut. This organism was not isolated from swabs from any other site on his body or from the other two astronauts. This organism (VPI strain no. 7726) was submitted to the Anaerobe Laboratory of VPI & SU for identification, and we determined that it had characterisitcs of P. niger . Because of the taxonomic importance of this coccus as the type species of the genus Peptococcus, we tried to isolate additional strains. This paper designates ATCC strain no. 27731 (VPI strain no. 7953) as the neotype strain and describes additional strains from eight different people.

MATERIALS AND METHODS

Cultures. The strains examined and their sources are listed in Table 4.

Media. Blood agar plates were made with brain heart infusion agar (Difco) with 4% laked sheep blood and 0.5% yeast extract added.

Loeffler's blood serum and meat infusion-peptone agar were made according to the procedure given by Hall (20). For Loeffler's blood serum, 1 part neutral beef broth containing 1% glucose, 1% peptone (Difco), and 0.5% NaCl was mixed with 3 parts of beef serum. Slants were made and sterilized by inspissation.

Meat infusion-peptone agar was prepared by making an overnight infusion from 500 g of ground beef and 1 liter of distilled water.

Table 4. Source of Peptococcus niger strains examined.

VPI No.[a]	Source
3206F	culture of drainage from rectal abscess; recieved from A. Balows, Lexington Clinic, Lexington, Ky.
6689C	culture from a pilonidal cyst; recieved from Corning Hospital, Corning, N.Y.
7726	culture isolated from a navel swab; recieved from Brown, Root, and Northrup, Houston, Texas
7953	navel swab, WM, Oct., 1972 (ATCC 27731[b])
8045	navel swab, TS, Nov., 1972
8046	navel swab, RG, Nov., 1972
8047	navel swab, GR, Nov., 1972
8878	vaginal area swab, TS, Aug., 1973
8893	navel swab, JH, Aug., 1973

[a]Virginia Polytechnic Institute and State University (VPI) number

[b]American Type Culture Collection (ATTCC) number

The infusion was boiled vigorously for 10 min. and strained through cheesecloth, and the meat was discarded. Peptone (20 g) and NaCl (5 g) were added; the mixture was boiled to dissolve the ingredients and then cooled. The pH was adjusted to 7.0 with NaOH, and 12 ml was pipetted into screw-capped tubes containing 0.25 g of agar and autoclaved at 15 psi for 15 min.

Chopped-meat agar was prepared as described previously for chopped-meat broth (29) except that agar to give a final 2% concentration was added to each tube before autoclaving and no meat particles were included in the final medium.

Carbohydrates tested. The carbohydrates tested were: adonitol, amygdalin, arabinose, cellobiose, dextrin, dulcitol, erythritol, esculin, fructose, galactose, glucose, lactose, maltose, mannitol, mannose, melezitose, melibiose, raffinose, rhamnose, ribose, salicin, sorbitol, sorbose, starch, sucrose, trehalose, and xylose.

Anaerobiosis. Blood agar plates were placed in anaerobe jars containing palladium catalyst, evacuated, and filled with 90% H_2 and 10% CO_2. Prereduced tube media were prepared as previously described (29). To reproduce Hall's (21) methods, we used alkaline pyrogallol (10) for maintaining anaerobiosis of blood agar slants and of the Loeffler coagulated blood serum.

Determination of %G+C. The percent guanine plus cytosine (%G+C) of the deoxyribonucleic acid preparations was determined by their thermal melting point (Tm) (43), using an automatic spectrophotometer (Gilford Instrument Laboratories). Escherichia coli B deoxyribonucleic acid was included in each spectrophotometer run as a standard.

Isolation from skin. Dry cotton swabs were vigorously rubbed on the skin surface to be tested and then rubbed onto a small area of a blood agar plate. This material was then streaked to obtain isolated colonies. The plates were incubated for 5 days in anaerobe jars. All black colonies were picked into chopped-meat broth (29). Cultures yielding Gram-positive cocci were streaked again to insure purity.

Characterization. Pure cultures were characterized by using media and methods previously described (29).

RESULTS AND DISCUSSION

Isolation from human navels. The navels of approximately 150 people were tested for the prescence of cocci that formed black pigment on blood agar plates. We isolated such organisms from the navels of five people. Two persons (one male, one female) were available for repeated culturing. We obtained repeated navel isolates from both of them at each of three samplings over a 12-month period. Swabs were obtained from skin surfaces at 40 different locations scattered completely over the body of these volunteers. Samples from W. M. were negative in all areas except the navel; one swab from the vaginal area of T.S. was positive in addition to the navel swab. These data indicate that black-pigmented cocci are present in the normal flora of the navels of a minority of the North American population and that these organisms are not distributed over the entire skin of these individuals. All navel strains appeared similar in cultural reactions and also appeared similar to two clinical isolates (Table 4) in the culture collection of the VPI Anaerobe Laboratory.

Characteristics of strains. The strains were obligately anaerobic, nonmotile, Gram-positive cocci. The cells from 24 hour peptone-yeast extract-glucose cultures were 0.3 to 1.3 μ_m in diameter; the average diameter was 0.7 μm. The cells occured singly and in pairs, tetrads, and irregular masses. No chains were observed.

On initial isolation, the colonies on blood agar plates were black, minute to 0.5 mm, circular with entire margins, convex, shiny, smooth, and nonhemolytic. After several laboratory transfers or freeze drying, black colonies on blood agar plates were no longer observed. These non-pigmented colonies were like the pigmented colonies except that they were white and translucent. However, both fresh isolates and strains that would no longer form black colonies on blood agar plates did form pigmented colonies repeatedly in meat infusion-peptone agar deeps and in chopped-meat agar roll tubes. These colonies in the agar were minute to 0.5 mm, black, and lenticular. As described by Hall (21) if the black colonies were exposed to air, they turned light gray. Growth in peptone-yeast extract broth was nonturbid with a slight smooth white or grayish-white sediment.

Surface colonies on anaerobic slants of chopped-meat agar were 1 mm, circular, entire, convex, opaque, and white to light gray. There was no growth on either Loeffler's coagulated blood serum or blood agar slants, using alkaline pyrogallol to establish anaerobiosis.

The following reactions were uniformly negative: digestion of gelatin, meat, milk; esculin, hippurate, and starch hydrolysis; production of indole and acetoin; production of lecthinase and lipase; reduction of nitrate; and utilization of lactate. All strains produced H_2S, NH_3,

and H_2, and weakly decomposed H_2O_2. No carbohydrate tested was fermented, but pyruvate was utilized. Gas was produced in peptone-yeast extract-glucose agar deeps. Peptone-yeast extract-pyruvate cultures were turbid with a smooth gray sediment. The fermentation products (average milliequivalents per 100 ml of replicate cultures of all eight strains) of P. niger in peptone-yeast extract-pyruvate medium were: acetic (3.5), isobutyric (0.3), butyric (1.2), isovaleric (0.6), and caproic (0.4) acids. Ocassionally, trace amounts of propionic, valeric, lactic, and succinic acids were produced.

The %G+C of the neotype strain is 50. The neotype strain is ATCC 27731 (VPI 7953 isolated from the navel of W. M.).

Differentiation from other anaerobic cocci. Production of caproate and no fermentation of carbohydrates differentiate P. niger from Peptostreptococcus anaerobius, the anaerobic Gram-positive coccus that it most closely resembles in cultural characteristics. Strains of P. anaerobius do not form black colonies on blood agar plates, even though they may form black colonies in glucose agar deeps. P. niger is the only anaerobic coccus that we have found to form black colonies in chopped-meat agar deeps as originally described by Hall (21).

Placement of the Name *Peptococcus anaerobius* (Hamm) Douglas on the List of *Nomina rejicienda*

INTRODUCTION

Peptococcus anaerobius (Hamm) Douglas was first described under the name *Staphylococcus anaerobius* by A. Hamm in 1912. A study of Hamm's publication indicates that the original characterization of this organism was based primarily on published reports of anaerobic staphylococci isolated by other authors, even though Hamm did mention the isolation of an anaerobic coccus. The currently accepted descriptions of this organism were not taken from the original publication by Hamm but from the description of *Staphylococcus anaerobius* by A. R. Prévot in 1933. Modern data and insight strongly suggest that the original description of *Staphylococcus anaerobius* Hamm was based on a very small number of generally variable and nondifferentiating characterisitics of atrains which very probably represented several species of anaerobic cocci. Provision 3 of the International Code of Nomenclature of Bacteria states that "...names applied to a group made up of two or more discordant elements, especially if these elements were erroneously supposed to form part of the same individual (*nomina confusa*)..." are to be placed on the list of nomina rejicienda. Therefore, it is requested that the Judicial Commission of the International Committee on Systematic Bacteriology issue an Opinion establishing the name *Peptococcus anaerobius* (Hamm) Douglas as a nomen confusum according to Provision 3 of the International Code of Nomenclature of Bacteria and placing it on the list of rejected names.

HISTORICAL BACKGROUND

Staphylococcus anaerobius Hamm (22) was first described in 1912. Hamm's description of Staphylococcus anaerobius is quite brief, and a translation from the German follows:

> 12. Staphylococcus anaerobius: I should like to combine here the obligately anaerobic, Gram-positive staphylococci which have been described up to now separately.
>
> First the species described by Wegelius as No. 35: small, round cocci of somewhat varying size, without capsule, Gram-positive. Yellowish, moderately raised colonies visible generally only after forty-eight hours, diffusing a weakly fetid odor. Encountered in the vagina in five cases. Isolated once by me from foul smelling pus of a Douglas abscess in a pure culture. Bondy reports to me personally about the finding of an anaerobic, Gram-positive staphylococcus from the pus of a fatal peritonitis puerperalis after abortion. Schottmüller mentions 'the not rare occurence' of an obligately anaerobic hemophilic staphylococcus.

Hamm's description thus was based slmost entirely on Wegelius' more detailed description of coccus No. 35. Apparently Hamm did not examine any of the strains isolated by Wegelius, Bondy, or Schotmüller. In addition, he did not present any of the characteristics of the organism he isolated from a human rectouterine abscess.

In 1907, Jungano (34) described strains of anaerobic staphylococci isolated from appendicitis, cystitis, and gangrenous infiltration of the perineum, but he failed to name the organism. Prévot isolated from an

infected tonsil an organism he believed to be the same as Jungano's anaerobic staphylococcus. He adopted the name Staphylococcus anaerobius from Hamm but based his description on Jungano's publication and on characteristics of the strain he had isolated from tonsils (51). Prévot also did not examine any of the strains isolated by Jungano, Hamm, Wegelius, Bondy, or Schotmüller.

A comparsion of these early descriptions of anaerobic staphylococci is given in Table 5. The colonial characteristics and the descriptions of growth in broth are summarized below.

Colonial characteristics. Staphylococcus anaerobius Hamm (22): yellowish, moderately raised colonies, emmitting a slight fetid odor (surface colonies); no description of agar deep colonies.

Wegelius' anaerobic coccus No. 35 (75): 1 mm, yellowish, round, elevated, smooth, with sinuate edge (surface colonies); 1 to 1.5 mm (8 to 10 days), trifoliate, but also lenticular, yellow, and often almost white (agar deep).

Jungano's anaerobic staphylococcus (34): large, round, biconvex with dark center and clear periphery (agar deep); no description of surface colonies.

Staphylococcus anaerobius (Jungano) Hamm as described by Prévot (51); lenticular (agar deep); no description of surface colonies.

Growth in broth. Staphylococcus anaerobius Hamm (22): not described.

Wegelius' anaerobic coccus No. 35 (75): slightly turbid with powder-like precipitate at the bottom and along the sides of the tube.

Jungano's anaerobic staphylococcus (34): growth was evident after 36 hours; flakes which soon sedimented to the bottom of the tube formed

Table 5. Characteristics of anaerobic staphylococci as described by Hamm, Wegelius, Bondy, Schottmüller, Jungano and Prévot.

Characteristic[a]	Hamm (22)	Wegelius (75)	Bondy (5)	Schottmüller (62)	Jungano (34)	Prévot (51)
cellular morphology	round small,varying size	small,round, varying sizes,[b] 0.5-0.8 μm diam. pairs and tetrads	staphylo-coccus-like	staphylo-coccus-like	cocci, singly, or in groups of 2 or 3	cocci in masses; 0.5-0.6 μm diam.
capsule	–	–	nr	nr	nr	–
flagella	nr	nr	nr	nr	nr	–
spores	nr	nr	nr	nr	nr	–
gram reaction	+	+	nr	nr	+	+
acid-fast	nr	–	nr	nr	nr	nr
anaerobic	+	+	+	+	+	+
killed by heating	nr	15 min. at 55	nr	nr	nr	10 min. at 80 30 min. at 60
pH range	nr	nr	nr	nr	nr	6-8
temp. of growth	nr	28-37	nr	nr	22-37	22-38
growth requirement	blood	nr	nr	blood	nr	nr
viability	nr	2-3 weeks	nr	nr	nr	2 months
gas production	nr	variable	nr	nr	–	–
indole production	nr	nr	nr	nr	nr	–
carbohydrate fermentation	nr	weak in fructose and peptone	nr	nr	c	d
action on milk	nr	none	nr	nr	nr	none
gelatin digestion	nr	nr	nr	nr	e	–
pathogenic	nr	–	nr	nr	for guinea pig and rabbit	for guinea pig and rabbit
toxin	nr	nr	nr	nr	nr	–
hemolysin	nr	nr	nr	nr	nr	–
source	pus from a rectouterine abscess	vagina, uterus	afebrile puerperal infection, peritoneal pus	nr	appendicitis, cystitis, gangrenous infiltration of perineum	tonsilitis

[a]Symbols: + = positive result; – = negative result; nr = result not reported.

[b]Wegelius (75) reported that after cultivation, the variation in size no longer appears.

[c]Jugano, in a later publication with Distaso (35), reported that his anaerobic staphylococcus was unable to ferment sucrose, glucose, dextrin, and lactose.

[d]Jugano (34) first reported weak gelatin liquefaction; later he and Distaso (35) reported negative gelatin liquefaction.

[e]Prévot (51) reported that his strains weakly fermented glucose and galactose. He mentioned that Jungano's strain did not ferment carbohydrates.

in the broth, and after 5 days the broth became clear.

DISCUSSION

In Hamm's (22) brief description of Staphylococus anaerobius, he stated: "Hier möchte ich die bisher ganz vereinzelt beschrieben obligat anaeroben, grampositiven Staphylokokken zusammenfassen." Because of this statement, and because of Hamm's failure to obtain and examine any of these strains or present the characteristics of the strain he isolated, we feel that Hamm could very well have been combining similar, but different, species of anaerobic cocci.

The following discussion indicates that these strains are different and suggests the species with which they are identical. The strains of Jungano must be considered in this discussion since Jungano's (34) characterization is the original basis of recent descriptions (13,52,59) of Peptococcus anaerobius. Since none of the strains isolated by Hamm, Wegelius, Jungano, Schotmüller, or Bondy exists today, comparsions can only be made by using the published descriptions.

Schotmüller's coccus. Hamm cited as the reference for the blood-requiring anaerobic staphylococcus the article, "Ueber bakteriologische Untersuchungen und ihre Methoden bei Febris puerperalis," published by Schotmüller (63) in April 1911. This article described a method for isolating anaerobic bacteria from cases of puerperal fever. Schotmüller stated that the addition of blood to the media was necessary for the isolation of an anaerobic staphylococcus. He gave no further description of this organism. In 1912, prior to Hamm's description of Staphylococcus anaerobius, Schotmüller (64) published the description of this

blood-requiring organism, named it Staphylococcus aerogenes, Schotmüller the 1911 article in his description of colony types of Staphylococcus aerogenes. He stated: "Die Kultur auf Blutagarplastilinplatte (München Med. Wochenshr., 1911., No. 15) lässt nach länger als 24-stündiger Bebrütungszeit (37°) verschiedner Grösse erkennen." Thus, the name Staphylo-aerogenes Schotmüller would have precedence over Staphylococcus anaerobius Hamm if the organisms are the same.

Wegelius' coccus No. 35. Wegelius' coccus No. 35 could be Peptococcus variabilis as described by Foubert and Douglas (16) in 1948. Foubert and Douglas (16) stated that "... in contrast to the uniform cell size of M. anaerobius [Staphylococcus anaerobius Hamm] the cell diameter of each individual strain in this group varies from 0.5 to 1.0 or 1.5 microns, and cells of all sizes may be present in a single culture." Wegelius (75) reported that the diameter of coccus No. 35 is 0.5 to 0.8 μm; for Wegelius' coccus No. 35, Heurlin (26) reported a diameter of 0.3 to 1.0 μm, with the smaller size predominating. Wegelius (75) stated that the cocci he described did show variation in cell sizes within a single culture.

Microccus variabilis (16) was found to liquefy gelatin. Wegelius (75), however, did not study the gelatin-liquefying abilities of his coccus. Nonliquefaction of gelatin was a characteristic reported by Jungano and Distaso (35) in 1910 and accepted by Prévot in 1933 in his description of Staphylococcus anaerobius. Peptococcus variabilis was distinguished from Peptococcus anerobius by varying cell sizes in a single culture and by gelatin liquefaction (13,16), but the name Peptococcus variabilis is considered synonymous with Peptococcus anaerobius

by Rogosa (59).

Wegelius (75) found that his coccus No. 35 produced acid from peptone and fructose. Occasionally a weak gas formation from fructose occurred. We have shown, by chromatographic analysis of fermentation products of several strains of Peptococcus variabilis (including the type strain, ATCC 14955), that this species is capable of fermenting fructose when 0.1% Tween 80 is present in the medium. The pH of the medium may not always be lowered. Results are shown in Table 6. Acetic acid is also produced from peptone-yeast extract medium, although not in as great a quantity as from fructose. Because of the varying cell sizes and the fermentation of fructose, we believe Wegelius' coccus No. 35 to be identical with Peptococcus variabilis (Foubert and Douglas) Douglas.

Jungano's anaerobic staphylococcus. In 1907, Jungano (34), while studying urinary infections in the laboratory of M. Metchnikoff at the Pasteur Institute, isolated and described an anaerobic staphylococcus. In 1910, Jungano and Distaso (35), who collaborated on the book Les Anaérobies while both were at the Pasteur Institute, further described Jungano's anaerobic staphylococcus. In this publication, they reported that Distaso had isolated Jungano's anaerobic staphylococcus from fecal material. In 1912 Distaso (12), who had studied the bacterial flora of fecal material in Metchnikoff's laboratory, published his description of Staphylococcus asaccharolyticus.

The only discrepancy that Distaso found between his organism and Jungano's was cell size. However, neither author listed the exact size of his organism. Distaso simply stated that Staphylococcus asaccharolyticus was twice as large as Jungano's anaerobic Staphylococcus.

Table 6. Chromatographic analysis of volatile acid products of *Peptococcus variabilis* (Foubert and Douglas) Douglas.[a]

VPI No.	source	pH		acetate[b]	
		fructose	PY[c]	fructose	PY[c]
5662	ATCC 14955 Foubert type	5.60	6.45	5.30	2.05
5663	ATCC 14956 Foubert	5.75	6.60	3.65	1.75
5187	Prévot 1884A	6.02	6.20	3.35	1.40
5467	Prévot 1469B	5.95	6.19	4.40	1.25
5753	Prévot 295	6.11	6.40	4.36	3.70
		6.15	6.40	4.40	2.75
5885A	5221336, Medical College of Virginia, Richmond	6.15	6.35	3.45	1.55
		6.12	6.15	3.65	1.85
6460B	672, Corning Hospital, Corning, New York	6.20	6.15	3.65	1.85
		6.15	6.20	3.65	2.45
7031	2565, Florida State Board of Health	5.51	6.51	6.10	1.20
		5.70	6.50	3.55	1.75
7096	TRW Hazelton Lab, Vienna, Virginia	6.22	6.32	3.30	2.30
		6.20	6.15	3.70	3.20
7101	TRW Hazelton Lab, Vienna, Virginia	5.70	6.12	4.25	1.05
7347	N-977-70A, Emory University Atlanta, Georgia	6.01	6.10	3.90	2.80
		6.10	6.35	3.65	2.35

[a]Cultural and chromatographic procedures as described in the Anaerobe Laboratory Manual (29).

[b]In milliequivalents per 100 ml culture.

[c]Peptone-yeast extract medium.

In 1933, Prévot (51), who had no strains of Staphylococcus asaccharolyticus, reported the cell size to be 1.0 to 1.2 µm. This value is twice the measurement of 0.5 to 0.6 µm which Prévot observed for his strain of Staphylococcus anaerobius isolated from tonsils. Prévot assumed from Distaso's description that the cells of Staphylococcus asaccharolyticus were twice as large as the cells of Jungano's anaerobic staphylococcus.

Table 7 gives the cell sizes of individual strains of Staphylococcus asaccharolyticus and Staphylococcus anaerobius isolated and identified by Prévot after 1933. The cell size measurements of these two species fall into overlapping ranges: 0.5 to 1.2 µm for Staphylococcus asaccharolyticus and 0.3 to 0.8 µm for Staphylococcus anaerobius. One must conclude that cell size is a somewhat variable characteristic.

In the original description of Staphylococcus asaccharolyticus, Distaso (12) reported that the species produced indole and emitted a bad odor. Jungano did not mention whether or not his staphylococcus produced an odor. Neither Jungano nor any of the other authors describing strains similar to anaerobic staphylococci reported indole reactions. Since Distaso and Jungano worked together, Distaso might have tested Jungano's strains before he wrote that Staphylococcus asaccharolyticus "...s'approche par sa biologie de celui isolé par Jungano."

Jungano's descriptions (34,35) of an anaerobic staphylococcus, however, are nearly identical with Tissier and Martelly's (72) and with Prévot's original description of Diplococcus magnus [Peptostreptococcus magnus (Prévot) Smith; Peptococcus magnus (Prévot) Moore and Holdeman (29)] except for cell size. Tissier and Martelly (72) reported that

Table 7. Cell size of *Staphylococcus asaccharolyticus* and *Staphylococcus anaerobius* strains isolated by A. R. Prévot.

Strain[a]	original identification	size in micrometers[b]
HD II	*S. asaccharolyticus*	0.6-0.7
266 D	*S. asaccharolyticus*	0.7-0.8
534 A	*S. asaccharolyticus*	0.8
600	*S. asaccharolyticus*	0.6-0.7
808 A	*S. asaccharolyticus*	0.8-1.0
998	*S. asaccharolyticus*	1.0-1.2
1813	*S. asaccharolyticus*	0.5
2229	*S. asaccharolyticus*	0.7-0.9
2480 A	*S. asaccharolyticus*	0.8-1.0
TR 7	*S. anaerobius*	0.5
390 B	*S. anaerobius*	0.5-0.6
681 B	*S. anaerobius*	0.5-0.7
1324	*S. anaerobius*	0.6
1469 B	*S. anaerobius*	0.5
1718	*S. anaerobius*	0.4
1793	*S. anaerobius*	0.4
2081	*S. anaerobius*	0.3
2253 B	*S. anaerobius*	0.4
2381	*S. anaerobius*	0.5
2342 B	*S. anaerobius*	0.5
2357 B	*S. anaerobius*	0.8
2529 B	*S. anaerobius*	0.8
2640	*S. anaerobius*	small cocci
2719 B	*S. anaerobius*	0.6
2767	*S. anaerobius*	0.8
2906 B	*S. anaerobius*	0.5
2991 C	*S. anaerobius*	0.4-0.7
3433	*S. anaerobius*	0.6
3493 A	*S. anaerobius*	0.8
3778	*S. anaerobius*	0.8
3889 A	*S. anaerobius*	0.6
3896 A	*S. anaerobius*	0.6
3956	*S. anaerobius*	0.6
4049	*S. anaerobius*	0.6
4094 B	*S. anaerobius*	0.6
4196 A	*S. anaerobius*	0.6
4217	*S. anaerobius*	0.6

[a] We are greatly indebted to A. R. Prévot for copies of his records. The strain number and identification are as given by Prévot.

[b] As reported by A. R. Prévot.

Diplococcus magnus anaerobius, which they isolated from putrefying butcher's meat, was difficult to distinguish from other cocci. However, in broth it appeared as a large coccus. Tissier and Martelly (72) failed to give an exact measurement of their organism. The size in micrometers was a characteristic added by Prévot (51) in 1933. Prévot did not study any of the strains isolated by Tissier and Martelly.

Descriptions of deep agar colonies of Jungano's coccus (34) and those of Diplococcus magnus anaerobius (Tissier and Martelly) (72) appear to be identical. Tissier and Martelly (72) reported that colonies were 1 to 2 mm in diameter with a granular surface and that they appeared to be formed of concentric circles with sucessively clearer zones and a dark center. Prévot's (51) description of Diplococcus magnus colonies is identical with that by Tissier and Martelly. Jungano (34) reported that the colonies of his anaerobic staphylococcus in deep agar were large, round, and biconvex with dark centers and clear peripheries.

Other characteristics given by Tissier and Martelly (72) and Prévot (51) correspond with those published by Jungano (34), as seen in Table 8. Jungano's organism could have been either Peptococcus magnus, Peptococcus asaccharolyticus, or some similar anaerobic coccal species.

CONCLUSIONS

From the above discussion, it is evident that Hamm's description was based on two, if not three, different species. Schotmüller's blood-requiring staphylococcus is Peptococcus aerogenes (Schotmüller) Douglas. Coccus No. 35 of Wegelius is, in all probability, Peptococcus variabilis

Table 8. Characteristics of Jungano's anaerobic staphylococcus, *Diplococcus magnus anaerobius* Tissier and Martelly, and *Diplococcus magnus* Prévot.

characteristic[a]	Jungano's anaerobic staphylococcus		*Diplococcus magnus anaerobius*, Tissier and Martelly (72)[d]	*Diplococcus magnus* Prévot (51)[e]
	Jungano (34)[b]	Jungano & Distaso (35)[c]		
cellular morphology	staphylococcus-like	small coccus	large coccus; singly, in pairs, masses, or short chains	large coccus; singly, in pairs, masses, or short chains 1.5-1.8 μm in diameter
Gram reaction	+	+	+	+
broth growth	turbid; flakes are deposited as sediment; broth becomes clear	turbid, becomes clear after 5 days	turbid; viscous sediment appears with clearing of broth	turbid; viscous sediment appears with clearing of broth
anaerobic	+	+	+	+
killed by heating	nr	nr	nr	1 hour at 60 5 min at 100
pH range	nr	nr	nr	5.5-8.5
optimum pH	nr	nr	nr	7.0
temperature of growth	22-37	nr	24-37	18-37
viability	2 weeks	2 months	nr	3 weeks
gas production	–	nr	–	–
carbohydrate fermentation	nr	–	–	–
gelatin digestion	very weak	–	–	–
action on:				
milk	nr	none	none	none
fibrin	nr	nr	none	none
egg white	nr	none	nr	nr
proteoses	nr	nr	nr	digested
ammonia production	nr	nr	+	+
indole production	nr	nr	–	–
pathogenic for	guinea pig and rabbit	guinea pig and rabbit	nr	not pathogenic
toxin	nr	–	nr	–
hemolysin	nr	nr	nr	–

[a]Symbols: – = negative result; + = positive result; nr = not reported.

[b]Isolated from appendicitis, cystitis, gangrenous infiltration of the perineum.

[c]Isolated from urinary tract and fecal material, in addition to earlier reported sources.

[d]Isolated from putrefying butcher's meat.

[e]Isolated from acute appendicitis.

(Foubert and Douglas) Douglas. The organisms isolated by Bondy and Hamm could be any of several anaerobic cocci.

There is also some question as to the identity of the organism that Prévot described as Staphylococcus anaerobius. His description was based on Jungano's report of an anaerobic staphylococcus and also on the characteristics of an organism he isolated from tonsilitis. Jungano could have been describing either Peptococcus asaccharolyticus (Distaso) Douglas, Peptostreptococcus magnus (Prévot) Smith (Peptococcus magnus [Prévot] Moore and Holdeman), or some other anaerobic coccus.

One must keep in mind that species in the genus Peptococcus are very similar in cellular morphology and cultural characteristics, even though they may be quite distinct biochemically (13,16,52,59). Early authors primarily used morphological and cultural characteristics in distinguishing one species from another. Because of the paucity of biochemical characteristics cited in these early descriptions of anaerobic cocci, both the purity of the strains and the validity of grouping several strains into a species can be questioned.

In the 8th edition of Bergey's Manual of Determinative Bacteriology, M. Rogosa (59) proposed that Peptococcus variabilis (Foubert and Douglas) Douglas, Peptococcus glycinophilus (Cardon and Barker) Douglas, and Peptostreptococcus magnus (Prévot) Smith be considered synonyms of Peptococcus anaerobius. Until additional data can be obtained, we feel that these should remain as separate species or, alternatively, that Peptococcus variabilis and Peptococcus glycinophilus be considered as later synonyms of Peptococcus magnus.

We believe that the name Peptococcus anaerobius is a nomen confusum.

Further, the specific epithet in this name is identical with that in the name for a different species of anaerobic cocci, Peptostreptococcus anaerobius (Natvig) Kluyver and van Niel. The confusion that results by retaining the same specific epithets in the names of two different species of anaerobic cocci which belong to two different genera whose names are very similar and have the same initial letter is directly contrary to Principle I of the International Code of Nomenclature -- the avoidance of confusion. In this particular case, it is quite important that confusion be avoided, for both organisms have clinical significance. For these reasons, we request that the Judicial Commission of the International Committee on Systematic Bacteriology issue an Opinion placing the name Peptococcus anaerobius on the list of nomina rejicienda.

Medium-Dependent Inhibition of *Peptostreptococcus anaerobius* by Sodium Polyanetholsulfonate in Blood Culture Media

INTRODUCTION

Sodium polyanetholsulfonate (SPS) is used in most blood culture bottles because it prevents coagulation (73) and eliminates the bactericidal action of blood (4,42,73). The major disadvantage with the use of SPS is its inhibition of growth of anaerobic cocci (27). Recently, it has been reported that SPS only inhibits one species of anaerobic cocci, *Peptostreptococcus anaerobius* (18,37). Unfortunately, *Peptostreptococcus anaerobius* is the most common anaerobic coccus isolated in clinical laboratories (18); and therefore, the use of SPS may drastically reduce the number of isolations from blood cultures.

Based on his preliminary observation, J. Mehl of Becton-Dickinson & Co. (Rutherford, N.J.) suggested that the sensitivity of *Peptostreptococcus anaerobius* to SPS might depend on the type of media used. In this paper, we compare the degree of SPS inhibition in several media and identify those compounds that increased the resistance of *Peptostretptococcus anaerobius* to SPS. In addition, we tested the conclusion of Graves et al. (18) that *Peptostreptococcus anaerobius* is the only species of anaerobic cocci that is inhibited by SPS.

MATERIALS AND METHODS

Bacterial strains. All bacterial strains tested for sensitivity to SPS were clinical isolates from the culture collection of the Anaerobe

Laboratory, Virginia Polytechnic Institute and State University (Table 9) as identified by L. V. Holdeman or W. E. C. Moore, according to procedures described in the Virginia Polytechnic Institute Anaerobe Laboratory Manual (29). Escherichia coli C and Serratia marcescens SM 29, two serum-sensitive strains (42,74), were gifts from W. H. Traub, Institut für Klinische Mikrobiologie und Infektionshygiene der Universität Erlangen-Nürnberg, West Germany.

Anaerobiosis. An anaerobic chamber, similar to that described by Aranki and Freter (2), manufactured by Coy Manufacturing Co., Ann Arbor, Mich., was used for all plating procedures, incubation, and storage of plates. The atmosphere in the chamber contained 5% CO_2, 10% H_2, and 85% N_2. All other anaerobic procedures were in CO_2-filled tubes as described in the Virginia Polytechnic Institute Anaerobe Laboratory Manual (29).

Sources of chemicals and media. Supplemented peptone broth (Vacutainer culture tube), quadtone, and SPS were supplied by Becton-Dickinson Co., Rutherford, N. J. The following were purchased from Baltimore Biological Laboratory, Cockeysville, Md.: brucella agar, Schaedler agar, Trypticase soy agar (11043), Columbia broth, Trypticase, and Gelysate. Brain heart infusion (BHI) agar, dextrose, yeast extract, proteose peptone, and gelatin were purchased from Difco Laboratories, Detroit, Mich. Agar (Fisher Scientific Co., Pittsburg, Pa.), NaCl (Fisher), casein (Fisher), bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.), defibrinated sheep blood (The Brown Laboratory, Topeka, Kan.), and rabbit serum (Pel-Freez Biologicals, Inc., Rogers, Ark.) were also used.

Table 9. Species of anaerobic cocci tested for sensitivity to SPS.

Species	No. of Strains
Peptostreptococcus anaerobius	26
Acidaminococcus fermentans	5
"Gaffkya" anaerobia	5
Peptococcus asaccharolyticus	5
Peptococcus magnus	16
Peptococcus prevotii	4
Peptococcus saccharolyticus	3
Peptostreptococcus parvulus	2
Streptococcus constellatus	5
Streptococcus intermedius	5
Streptococcus morbillorum	5
Veillonella alcalescens	1
Veillonella parvula	3

General media. Prereduced chopped meat carbohydrate broth, anaerobic dilution fluid, and pyruvate broth with 0.025% Tween 80 were made as described previously (29). The pH of all media used for plates was adjusted to pH 7.2. All media were sterlized by autoclaving (15 min. at 15 lb/in^2. Defbrinated sheep blood (5%) was used for blood agar plates. The plated media were dried aerobically in an incubator at 37 C and then stored in the anaerobic chamber overnight.

Composition of supplemented peptone broth. Commercially prepared supplemented peptone broth (B-D Vacutainer culture tube) contained the following ingredients: quadtone (a blend of peptones and yeast extract), 2%; gelatin, 1.2%; NaCl, 0.5%; dextrose, 0.5%; sodium bicarbonate, 0.22%; magnesium sulfate, 0.02%; sodium phosphate, dibasic, 0.014%; adenine, 0.01%; glutamine, 0.01%; p-aminobenzoic acid, 0.005%; glutamic acid, 0.005%; proline, 0.005%; SPS, 0.03%; L-cysteine, 0.039%; hemin, 0.005%; nictinoamide adenine dinucleotide, 0.000125%; menadione, 0.0002%; guanine, 0.00015%; cocarboxylase, 0.00015%; and vitamin B_{12}, 0.000005%. For supplemented peptone agar plates, the last seven ingredients were added as a filter-sterilized solution to an autoclaved solution of the other components plus 1.5% agar.

Quadtone basal medium. For most of the experiments, many of the ingredients of supplemented peptone were not necessary; therefore, a simplified medium containing quadtone (2.0%), NaCl (0.5%), dextrose (0.5%), and agar (1.5%) was used. Quadtone was replaced by yeast extract (0.5%) and either proteose peptone (2%), Trypticase (1%), or Gelysate (2%) for certain tests.

Sensitivity to SPS. Thirteen species of anaerobic cocci (Table 9)

were tested for sensitivity to SPS on the following five agar media: BHI blood, brucella blood, Columbia blood, Schaedler blood, and supplemented peptone. Cultures were tested for sensitivity to SPS on agar plates inoculated with a Steers replicator (69). The inoculum was an overnight culture grown in pyruvate-Tween broth and then anaerobically diluted to an optical density of 0.2 at 650 nm (18mm light path, B&L Spectronic 20). The replication procedure was performed in the anaerobic chamber, and all plates were incubated in the chamber at 37 C for 48 hours. Organisms were considered sensitive when they failed to grow or showed diminished growth in comparsion to the control plate without SPS.

Minimal inhibitory concentrations (MIC) of SPS for 16 strains of Peptostreptococcus anaerobius were determined as described above. The media used were quadtone base plus 5% blood and 1.2% gelatin. The MIC was defined as the lowest concentration of SPS that inhibited all growth.

Serum sensitivity studies. Aerobic quadtone broth (with and without 1.2% gelatin), to which 10% fresh human serum and varying concentrations of SPS had been added, was inoculated with approximately 10^3 cells/ml of log-phase cultures of either E. coli C or S. marcescens SM 29. Both strains were killed by the human serum used in our tests. Turbidity was recorded after overnight incubation at 37 C.

Growth in simulated blood cultures. Dilutions estimated to contain ten cells, on the basis of a Petroff-Hauser microscopic count of a log-phase culture of two serum-sensitive strains (E. coli C and S. marcescens SM 29) were injected into B-D Vacutainer culture tubes which contained supplemented peptone broth (45 ml) and freshly drawn human blood (5 ml). The tubes were incubated at 37 C until growth appeared.

Several strains of Peptostreptococcus anaerobius were also tested by the above procedure for ability to grow in the supplemented peptone broth without blood.

RESULTS

Medium dependence of SPS inhibition. Of the 13 species of anaerobic cocci tested (Table 9), Peptostreptococcus anaerobius was the only species that was inhibited by 0.1% SPS. The toxicity of SPS for Peptostreptococcus anaerobius was dependent on which medium was used for the test (Table 10). SPS was much less toxic in supplemented peptone (Vacutainer culture tube medium) and BHI media than in the other media we tested (Table 10).

Media components that prevented SPS inhibition. The major components of supplemented peptone medium were tested to determine what was responsible for the lack of SPS inhibiiton in that medium. Quadtone, a blend of peptones and yeast extract, was not effective. Gelatin, the other major ingredient, was protective. Addition of 1.2% gelatin to the quadtione basal medium allowed 23 of 24 strains of Peptostreptococcus anaerobius to grow in the presence of 0.1% SPS, and 0.6% gelatin protected 10 of 24 strains. The effect of gelatin was not dependent on the media used. Addition of 1.2% gelatin also protected Peptostreptococcus anaerobius from SPS in Schaedler, brucella, and Columbia blood agar (Table 11). Gelatin was effective regardless of whether it was autoclaved with the media or added afterwards as a filter-sterilized solution.

Proteose peptone appeared to be responsible for the lower toxicity

Table 10. Sensitivity of 24 strains of Peptostreptococcus anaerobius to SPS on several agar media.

Media	No. of strains inhibited	
	0.05% SPS	0.1% SPS
Supplemented peptone agar	1	3
BHI agar	7	15
BHI blood agar	7	15
Schaedler blood agar	21	24
Trypticase soy agar	23	23
Brucella blood agar	24	24
Columbia blood agar	24	24

Table 11. Reversal of SPS toxicity by gelatin for 24 strains of *Peptostreptococcus anaerobius* in various media.

Base media	No. of strains inhibited			
	0.05% SPS	0.05% SPS + 1.2% gelatin	0.1% SPS	0.1% SPS + 1.2% gelatin
Schaedler	24	1	24	4
Brucella	24	1	24	10
Columbia	24	0	24	6

of SPS in BHI (Difco Laboraotries, Detroit, Mich.) medium. In a medium containing 2% proteose peptone, 13 of 26 strains of Peptostreptococcus anerobius grew in the presence of 0.1% SPS. We did not test brain and heart infusions, which are the other major ingredients of BHI, because they were not available commercially. The activity of proteose peptone was sufficient, however, to account for the effect of BHI.

Other compounds tested. Addition of blood, serum, and bovine serum albumin to media did not affect SPS toxicity. However, casein did protect Peptostreptococcus anaerobius from SPS (Table 12). Trypticase, a pancreatic digest of casein, was not protective, nor was Gelysate, a pancreatic digest of gelatin (Table 12).

Extent of protection by gelatin. To measure the extent of protection provided by gelatin, we determined the MIC of SPS in a simple quadtone agar medium with and without 1.2% gelatin. The 16 strains tested had MIC values of less than 0.006% SPS in the absence of gelatin, and gelatin raised the MIC values by an average of 64-fold (Table 13). The three strains that were most sensitive to SPS had MIC values of 0.125% SPS in the presence of gelatin. However, in supplemented peptone broth, which contains 0.03% SPS and 1.2% gelatin, all three strains grew to maximum turbidity within 48 hours from inocula of approximately 10 cells per 50 ml of broth. This may have been due to the better growth conditions of the complete medium.

Effect of gelatin on beneficial effects of SPS. SPS is used in blood culture media to eliminate the bactericidal effect of human blood for serum-sensitive organisms. Because gelatin essentially eliminated the inhibitory effect of SPS on Peptostreptococcus anaerobius, we

Table 12. Effect of proteins other that gelatin on reversing the toxicity of SPS for Peptostreptococcus anaerobius.

Additives to base media	no. of strains tested	no. of strains sensitive
Casein, 1.2%	25	9
Casein, 2.4%	25	0
Bovine serum albumin, 1.2%	21	21
Rabbit serum, 20%[a]	25	24
Rabbit serum, heat inactivated, 20%[a]	25	22
Defibrinated sheep blood, 20%[a]	25	24

[a]This concentration of blood was equal to a protein concentration of 1.2% in the medium (68).

Table 13. Minimal inhibitory concentration of SPS for 16 strains of Peptostreptococcus anaerobius on quadtone plate medium.

Media	Minimal Inhibitory Concentration (Final % of SPS in media)									
	<0.00075	0.0015	0.003	0.006	0.0125	0.025	0.05	0.1	0.2	>0.2
base medium with 5% blood	6[a]	3	5	2	0	0	0	0	0	0
base medium with 5% blood plus 1.2% gelatin	0	0	0	0	3[b]	0	2	6	3	2

[a]Number of strains having the minimal inhibitory concentration.

[b]These strains were not inhibited by 0.03% SPS in B-D Supplemented peptone broth which contained gelatin.

considered that gelatin might also eliminate the beneficial effect of SPS. However, this did not appear to be so since two serum-sensitive organisms, E. coli C and S. marcescens SM 29, grew from inocula of approximately 10 cells/50 ml in the Vacutainer culture tube (0.03% SPS, 1.2% gelatin) in the presence of 10% fresh human blood.

Gelatin did have some effect on the ability of SPS to eliminate serum killing. When the two serum-sensitive strains were grown in a medium containing 10% human serum, the amount of SPS required to allow growth was increased fourfold by addition of 1.2% gelatin (Table 14). However, in the presence of gelatin the amount of SPS required for protection from serum killing (0.0125 to 0.006%) was still much less than the amount of SPS currently used in blood culture media (0.03 to 0.05%).

DISCUSSION

Our results are in agreement with the conclusion of Graves et al. (18) that SPS only inhibits Peptostreptococcus anaerobius and does not inhibit other common clinical isolates of anaerobic cocci. However, we have not tested a large enough number of strains of each species to detect possible strain variation. The SPS disk test for identification of Peptostreptococcus anaerobius (18) should prove useful for clinical laboratories, but larger scale tests will be necessary to document the accuracy of the test. Our study demonstrates that the test should be standardized with a single medium such as the Schaedler agar used by Graves et al. (18). Media for use with the SPS disk test should not contain proteose peptone, gelatin, or casein. Schaedler medium does not contain these ingredients.

Table 14. Protection of two serum sensitive strains (*E. coli* C and *S. marcescens* SM 29) in medium containing SPS and gelatin.

strain[a]	additives to base medium plus 10% serum	minimal effective concentration of SPS (final % of SPS in media)
E. coli C	None	0.003
	1.2% gelatin	0.0125
S. marcescens SM 29	None	0.0015
	1.2% gelatin	0.006

[a]Inoculum was approximately 10^3 cells/ml.

It is not yet known how SPS inhibits growth of Peptostreptococcus anaerobius. This makes it difficult to explain how gelatin and casein overcome the inhibition. The activity of these proteins seems to be related to their secondary or tertiarty structure, since their pancreatic digests, consisting of single amino acids and short peptides, were not active. The activity of proteose peptone may result from its content of large peptides ("proteoses") which are probably derived from gelatin: both proteose peptone and gelatin have an unusually high glycine content of 23 to 25%. SPS may be inactivated by binding to portions of gelatin and casein molecules just as it is known to bind to the serum proteins responsible for serum killing (73). However, gelatin only increased by fourfold the concentration of SPS needed to neutralize the bactericidal effect of human serum, whereas it increased 64-fold the concentration needed to inhibit Peptostreptococcus anaerobius.

Whatever its mechanism of action, gelatin appears to be a useful addition to blood culture media since it allows growth of Peptostreptococcus anaerobius while at the same time it does not prevent SPS from neutralizing the bactericidal effect of blood. Additional simulated and clinical blood culture studies should be performed to confirm these results because SPS and serum sensitivity might vary among strains, fresh clinical isolates might behave somewhat differently than our stock cultures, and the bactericidal activity of blood from different sources might vary as well.

CONCLUSIONS

This thesis represents an attempt to solve three of the taxonomic or growth problems associated with species in the genera Peptococcus and Peptostreptococcus. In one study, strains of Peptococcus niger were isolated, the description of this organism was emended to include additional characteristics, and a neotype strain was deposited with the American Type Culture Collection, Rockville, Maryland. In a second study, a proposal for rejecting the name Peptococcus anaerobius was made, and if the Judicial Commission of the International Committee on Systematic Bacteriology accepts this proposal, the name Peptococcus anaerobius should no longer be confused with the name Peptostreptococcus anaerobius. At the present time the Judicial Commission has not acted upon this request. In a third study, gelatin and proteose peptone were found to protect Peptostreptococcus anaerobius from inhibition by sodium polyanethol sulfonate (SPS). Peptostreptococcus anaerobius is frequently isolated from blood cultures (18), and the addition of gelatin to blood culture media containing SPS should be potentially helpful in recovering Peptostreptococcus anaerobius from blood cultures. Because media containing either gelatin or proteose peptone affects the inhibition of growth of Peptostreptococcus anaerobius by SPS, media containing either of these substances should not be used in the SPS test developed by Graves et al. (18) for identification of Peptostreptococcus anaerobius.

Even though some problems were solved, other problems were revealed. If the name Peptococcus anaerobius is rejected as a nomen confusum then, what would be the name of those anerobic cocci which conform to the description of Peptococcus anaerobius or those species which are

considered synonyms with Peptococcus anaerobius? Preliminary results have indicated that this group of anaerobic cocci might not be a homogenous group but rather might be two distinct groups. Additional examination of the characteristics of numerous strains and DNA hybridization studies should clarify whether or not there are indeed two distinct groups. For the present time, those species which have been considered to be synonymous with Peptococcus anaerobius should be regarded as separate species: Peptococcus magnus, P. glycinophilus, and P. variabilis.

Another problem concerning the anaerobic cocci is that of pigmentation. Rogosa (60) stated that "dark or black colony formation is not an unusual feature among the distinctly different species of Peptococcus, as well as Peptostreptococcus." Rogosa believes that these black-pigmented colonies are simply mutants among a large non-pigmented population. Holdeman and Moore (29) have also reported that strains of Peptostreptococcus anaerobius and P. micros may have black colonies depending on the medium in which they were grown. However, we found that all strains of P. niger we tested consistently had black colonies in chopped-meat agar deeps. We, therefore, do not believe that this pigment is a result of spontaneous mutatuion. The pigmentation found in this and other species of anaerobic cocci probably is the result of H_2S production in media containing sulphur compounds.

These studies were undertaken to clarify some of the taxonomic problems associated with the anaerobic cocci. However, continued research, specifically DNA hybridization studies, is needed to fully understand the taxonomic relationships among the anaerobic cocci.

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Taxonomic Studies of Two Species of Peptococci and Inhibition of *Peptostreptococcus anaerobius* by Sodium Polyanethol Sulfonate

by

Susan Emily Holt West

(ABSTRACT)

Three problems associated with the taxonomy and growth of anaerobic cocci belonging to the genera *Peptococcus* and *Peptostreptococcus* were studied. One study involved the designation of the neotype strain of *Peptococcus niger*, the type species of the genus *Peptococcus*. The type strain had been lost, and there were no extant strains of this taxonomically important species. Several strains of anaerobic cocci isolated from the navels of several human subjects were found to be identical with the original description of *Peptococcus niger*. Therefore, an emended description of this species was published. Another study involved the taxonomic status of *Peptococcus anaerobius*. A search of the early descriptions of *Peptococcus anaerobius* revealed that the original description of the species was probably based on descriptions of several different anaerobic cocci. Consequently, a request has been made of the Judicial Commission of the International Committee on Systematic Bacteriology to reject the name *Peptococcus anaerobius* as a *nomen confusum*. A third study involved the inhibition of growth of *Peptostreptococcus anaerobius* by sodium polyanethol sulfonate (SPS). Gelatin and proteose peptone were found to protect *Peptostreptococcus anaerobius* from inhibition of growth by SPS, and, therefore, are useful additions to blood culture media for the purpose of enhancing isolation of *Peptostreptococcus anaerobius* from blood cultures. Numerous strains of other anaerobic cocci were also tested for sensitivity to SPS.