UMEÅ UNIVERSITY ODONTOLOGICAL DISSERTATIONS
No. 7
From the Department of Oral Microbiology,
University of Umeå, Sweden

BACTERIOLOGICAL STUDIES OF NECROTIC DENTAL PULPS

by
GÖRAN SUNDQVIST

Umeå 1976

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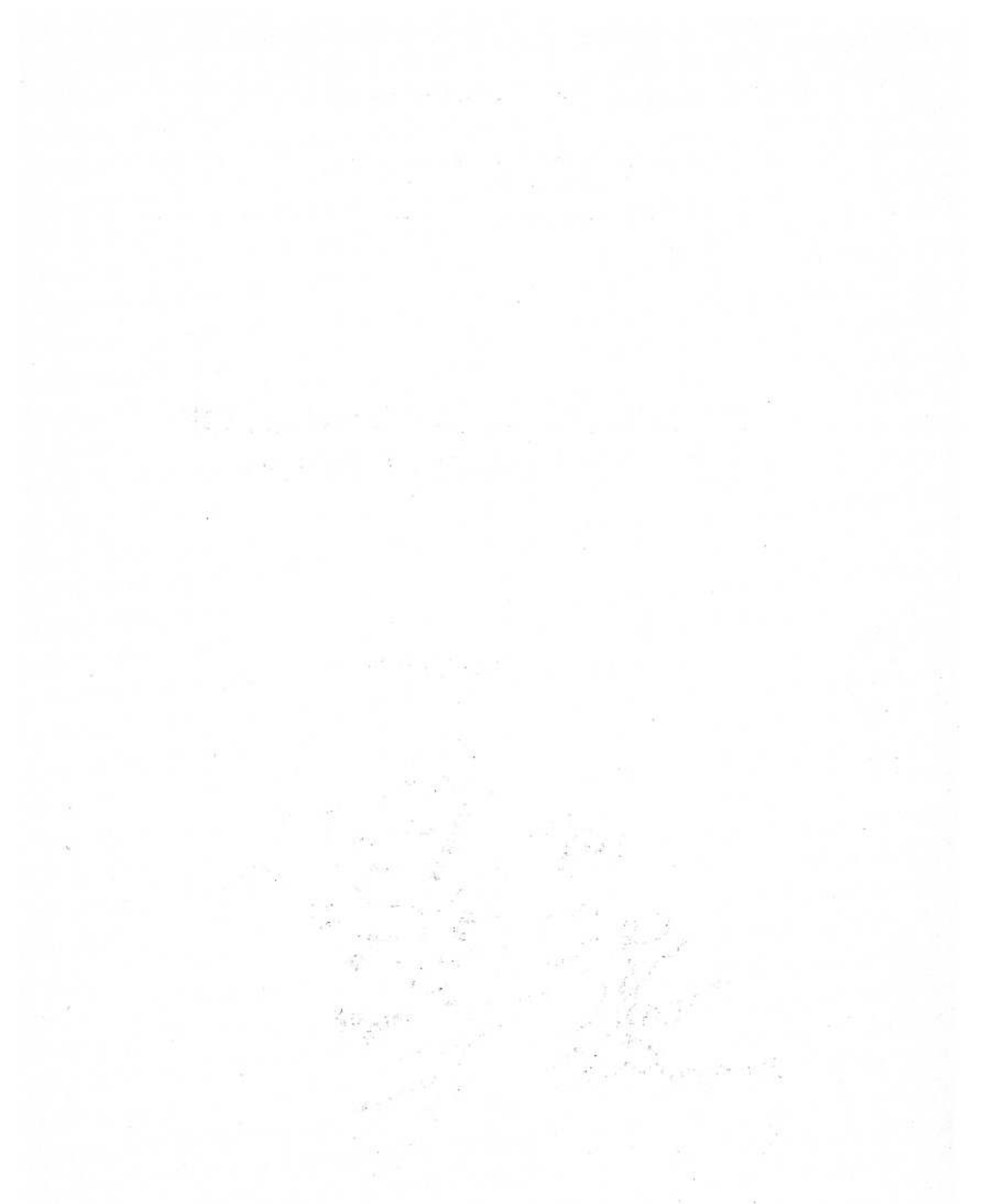

Figure on the cover. Bacteria in purulent pulp. Fig. 9 in Miller, W.D. 1894. An introduction to the study of the bacterio-pathology of the dental pulp. The Dental cosmos 36:505-528.

CONTENTS

I. INTRODUCTION

Since 1894, when W.D. Miller observed bacteria in inflamed pulp tissues, microorganisms have been considered important for the development of inflammation in the dental pulp. However, the ultimate importance of bacteria was not demonstrated until Kakehashi et al. (1965) showed that pulp necrosis and periapical bone destruction only developed in conventional and not in germ-free rats when the pulp chambers were kept open to the oral cavity.

In humans a higher incidence of periapical destruction after endodontic treatment occurs when bacteria have been isolated from the root canal just before obturation than if no bacteria were found (Zeldow and Ingle, 1963: Engström et al., 1964: Oliet and Sorin, 1969). However, a convincing correlation between the presence of bacteria in the root canal and the development of periapical destruction has not so far been demonstrated (Sommer and Crowley, 1940: Morse and Yates, 1941: Macdonald et al., 1957: Engström and Frostell, 1961).

In the present study bacteria could be isolated from the necrotic pulps of teeth only when periapical destruction was present. Therefore it is suggested that the isolated bacteria include the etiological agents of pulp disease involving pathological changes in the periapical region.

In the search for the etiological agents of pulp disease samples for bacterial culture have often been taken from the root canals of teeth with caries or restorations (Leavitt et al., 1958: Winkler and van Amerongen, 1959: Shovelton and Sidaway, 1960: Crawford and Shankle, 1961). When samples are taken from the pulp of teeth with intact crowns it is possible to avoid picking up bacteria from carious lesions or saliva and there is a higher probability that the isolated bacteria have importance for the pathological changes in the periapical region. Previous studies on necrotic pulps of teeth with intact pulp chambers are summarized in Table I:1. Most

of the isolated microorganisms are normal inhabitants of the oral cavity but they are recovered in various frequencies in the different studies. This could be explained by differences in bacteriological technique. Initial cultivation of the sample in broth which has been performed in some of the studies(Macdonald et al., 1957: Chirnside, 1957) increases the likelihood of recovering only the fast-growing bacteria when the sample contained a mixture of bacterial species. Another reason for the variation is that the characterization of isolated strains has been limited and that different criteria have been used for identification.This might explain the high recovery of corynebacteria and micrococci in the study of Brown and Rudolph (1957). Their recovery of Treponema was due to the fact that special precautions were taken for the isolation of these demanding microorganisms (Hampp, 1957).

The techniques have not been favourable for the recovery of anaerobic bacteria. Anaerobes made up 24 per cent of isolated strains in the study of Brown and Rudolph (1957) and 32 per cent in the study of Macdonald et al. (1957). In the study of Möller (1966) anaerobic bacteria were isolated from most infected root canals and made up 74 per cent of all isolated strains. All the studies cited in Table I:1 have been performed with techniques that are suboptimal for the isolation of anaerobic bacteria. However, advanced anaerobic techniques have recently been used in two studies on the bacterial flora in necrotic pulps of intact teeth (Kantz and Henry, 1974: Wittgow and Sabiston, 1975).

The PRAS (prereduced, anaerobically, sterilized) technique introduced by Hungate (1950) and simplified and further developed by Moore (1966) has facilitated the isolation of anaerobic bacteria (Spears and Freter, 1967: McMinn and Crawford, 1970: Berg and Nord, 1973). When using the PRAS-technique with roll tubes for growing samples from the gingival crevice Gordon et al. (1971) found that 70 per cent of the total microscopic count was cultivable. The corresponding figure was 20 per cent when plates were inoculated in air and incubated in conventional anaerobic jars. Recently Fulghum et al. (1973) reported that bacteria could be recovered from 75 per cent of root canals when PRAS-media were used and from 42 per cent when samples were cultivated in thioglycolate broth. Wittgow and Sabis-

ton (1975) used PRAS roll tubes in their study on necrotic pulp of intact teeth.

A further technical improvement is the use of an anaerobic glove box (Socransky et al., 1959: Rosebury and Reynolds, 1964), in which bacteria are protected from oxygen during isolation and cultivation. The atmosphere in the box is usually a mixture of nitrogen, carbon dioxide and hydrogen. Using an anaerobic glove box Aranki et al., (1969) were able to significantly increase the recovery of bacteria from the contents of mouse caecum as compared to the results obtained in an earlier study using the PRAS-technique with roll tubes (Spears and Freter, 1967). When samples from the gingival crevice were grown in the glove box the viable count was 50 per cent of the microscopic count, as compared to 24 per cent when all procedures were performed in the same way except that the plates were inoculated outside the glove box (Aranki et al., 1969).

Obviously the etiology of pulp disease cannot be clarified without an optimal technique for the isolation of bacteria. In the present study samples were obtained from infected root canals of non-vital teeth with intact crowns for culture of anaerobic, facultatively anaerobic and aerobic bacteria. Special precautions were taken to obtain low redox-potential during the handling of specimens for anaerobic culture. A surprisingly high yield of anaerobic bacteria was obtained. More than 90 per cent of the isolated strains were anaerobic.

Anaerobic as well as facultatively anaerobic bacteria isolated from infected root canals have until recently been characterized only by morphology and reaction to the Gram stain. In the present study as well as in the studies of Kantz and Henry (1974), and Wittgow and Sabiston (1975) all strains were characterized by methods described by Holdeman and Moore (1972) and identified as to the species level. Numerical taxonomic analysis was used in this study for evaluating the composition of the bacterial flora of the infected pulps in relation to the clinical condition of the teeth.

Table I:1

Microorganisms isolated from root canals of teeth with intact pulp chamber walls and necrotic pulp tissues.

	Authors	Mac-donald et al. 1957	Brown and Rudolph 1957	Chirnside 1957	Engström and Frostell 1961	Möller 1966	Bergenholtz 1974
	Number of cases	46	70	28	36	35	84
	Number of positive samples	38	59	15	21	29	54
	Total number of strains	71	114	39	42	83	218
Anaerobic bacteria	Anaerobic gram + cocci	12		9	8	16	27
	Veillonella			1	2	3	5
	Bacteroides	5		8	1	9	62
	Fusobacterium	2	3		11	6	26
	Vibrio	1					
	Treponema		14				
	Anaerobic gram + rods	3		4	4	25	48
	Leptotrichia					2	
Facultative and aerobic bacteria	α-hem streptococci	17	32	7	5	7	29
	β-hem streptococci			2		1	
	γ-hem streptococci	3		1	1	5	2
	Enterococcus				2	3	3
	Micrococcus	6	19				
	Staph. epidermidis	6					
	Staph. aureus	4		1			
	Neisseria	4	3	1	2		3
	Lactobacillus				3	3	
	Corynebacterium		28		1	1	8
	Coliform. bacteria	1	1				
	Aerobic gram - rods			4			
	Aerobic gram + rods	4		1	2		
	Pseudomonas	2	1				
	Yeast, molds	1				1	
	Unidentified		12				5
	Lost after initial isolation		34				
	Transport medium					+	+
	Initial cultivation in broth	+	+	+	+	+	+
	Initial cultivation on solid media		+		+	+	+

Clinical material

In 27 patients between 12 and 60 years of age thirty-two single rooted teeth with necrotic pulps and intact pulp chamber walls were studied. The teeth were free from caries as well as from restorations and were without palatinal enamel invaginations. Loss of vitality of the pulp had occurred as a result of impact injury. Teeth where the trauma had caused fractures of the root or crown, were not included. Neither teeth with a lowering of the marginal bone level of more than 3 millimeters, or teeth with pathological gingival pockets or sinus were accepted. The clinical examination also included registration of clinical symptoms such as pain and swelling. Radiographic examination was performed using a modified long cone technique (Oralix, Philips; film-focus distance 25 cm) with Kodak ultra-speed (24 by 36 mm) film and a filmholder (Eggen, 1974). Radiographs were taken in at least two projections, and examined by the author, before the bacteriological sampling, and by a roentgenologist (Dr. M. Hedin), who was unaware of the bacteriological findings. The two examiners agreed on the interpretations of the radiographs in all cases. The time that had elapsed since the trauma was noted if the patient was sure of it.

Chemicals

Palladium black was purchased from Engelhard Industries Ltd., High Holborn, London, England. Palladium chloride was from British Drug Houses Ltd., Poole, Dorset, U.K. who also delivered arabinose, cellobiose, esculin, glucose, mannitol, mannose, salicin, trehalose, xylose, raffinose, inositol, inulin, melibiose, rhamnose, ribose, sorbose, glycerol, pyruvic acid, L-threonine, cysteine hydrochloride, resazurin, indigo carmine, phenosafranine, benzyl viologen and sodium formaldehyd-sulfoxylat. Fructose, maltose, sorbitol, methylene blue and crystal violet were obtained from Merck A.G., Darmstadt, West Germany. Sucrose and lactic acid were obtained from Mallinckrodt Chemical Works, St. Louis, Ill., USA. Soluble starch and gelatin were from Fisher Scientific Company, Fair Lawn, N.J., USA. Dulcitol, lactose, melezitose were

from Baltimore Biological Laboratory, Cockeysville, Md, USA, who also delivered brain heart infusion broth (11059), mycoplasma broth base (11458), mycoplasma agar base (11456), trypticase soy broth (11768). Neutralized bacteriological proteose peptone (L46), tryptone soy agar (CM 131), yeast extract (L21), bacteriological charcoal (L9) and skimmed milk powder (L31) were from Oxoid Limited, London, England. Agar base (0140-01), Rogosa SL agar (0480-01), tryptose (0124-01), oxgall (0128-01) and potassium tellurite (0384-13) from Difco Laboratories, Detroit, Mich., USA. Trypan blue was from Chroma-Gesellshaft, Schmid & Co., Stuttgart, West Germany. Cocarboxylase, hemin, menadione were obtained from Sigma Chemical Co., 350 Dekalbs., St. Louis, Ill., USA. TEM-4T (diacetyl tartaric ester of tallow monoglycerides) was obtained from Witco Chemicals Organics Division, New York, N.Y., USA. Of antibiotics used in the media benzyl penicillin sodium was obtained from AB Kabi, Stockholm, Sweden; polymyxin B sulphate from Novo industri A/S Copenhagen, Denmark; nalidixic acid from Winthrop laboratories, Newcastle Upon Tyne, England and streptomycin sulphate from Glaxo Laboratories Ltd., Greenford, Middlesex, England. In the standard solution in gas chromatography; acetic, propionic, pyruvic, isobutyric, butyric, isovaleric, valeric, isohexanoic, hexanoic, heptanoic and succinic acids were from British Drug Houses. Lactic acid was from Mallinckrodt. 2-metyl valeric, 3-metyl valeric and 4-metyl valeric acids were from Pfaltz and Bauer, inc., Glushing, N.Y., USA.

Anaerobic glove box

The box used in this study was constructed along the lines of that deviced by Aranki et al. (1969). It was made of 2 mm steel plate with welded seams (John Bass Ltd., Crawley, Sussex, England). The dimensions were 900 mm long, 770 mm deep and 620 mm high. The window was made of 10 mm Perspex sheet and the dimensions were 520 mm by 880 mm. There were two glove ports in the front panel with an outside diameter of 25 cm. Neoprene rubber gloves (15 mil.) were used (Germfree Laboratories, inc., Miami, Fd., USA). The box had two round airlocks. One larger lock mounted on the right was made of 3 mm aluminium, 450 mm long by 300 mm in diameter. The smaller lock on the left was made of 5 mm perspex 280 mm long by 150 mm in diameter. A vacuum pump with a capacity of 75 Liters/min was used (Speedivac, model ES 75, Edward's High Vacuum Ltd., Manor Royal, Sussex, England).

The gas mixture used in the box was 10 per cent hydrogen and 5 per cent carbon

dioxide in nitrogen. Inside the box was a fan (type Microvent, E.T.R.I., Elfa AB, Solna, Sweden) which forced the box atmosphere through two trays of stainless-steel screening, each filled with 100 grams of catalyst pellets of palladium coated aluminia (Engelhard industries Ltd., High Holborn, London, England), and one tray containing 300 grams of reusable silica gel desiccant (Blau-gel, W.R. Grace AB, Helsingborg, Sweden). The trays, placed above each other, were 100 by 250 mm and exchangeable for rejuvenation. This system continuously circulated the atmosphere and removed traces of oxygen by catalytic reaction with hydrogen in the gas (Rosebury and Reynolds, 1964; Aranki et al., 1969) and kept the relative humidity between 30 per cent and 50 per cent. A ventilator for removing particles from the chamber atmosphere was also installed (Steril-Ventilator, Stora Kopparberg AB, Sweden).

The incubator, 250 by 400 by 500 mm, was made of plywood and insulated with styrene foam blankets. A temperature regulator (type Shinko NC-120-R/R, Elfa AB, Solna, Sweden) with a "resistance giver" (Pt 100 Ω) kept the temperature constant in the incubator within ±1 C. The atmosphere also passed over papers (Whatman MM 3, H Reeve Angel, Co. Ltd., London, England) saturated with lead acetate to absorbe hydrogen sulfide formed by the bacteria. One electrical control panel outside the chamber contained a transformer which reduced the initial voltage of 220 V to 12 V. Two leads, one with the voltage of 220 V and the the other with 12 V, from this outer panel were connected with another control panel inside the chamber. The lower voltage (12 V) was used for two inoculation loops and a coil of resistance wire which served to "flame" the rim of test tubes and flasks. The loops and the coil were connected with a foot switch. The inoculation loops were made of 30 cm resistance wire (Kanthal A 0.4 mm diam) soldered to thin flexible electrical cords. Each soldering was covered with a 20 cm Pyrex capillary tube with 10 cm of the resistance wire uncovered. The Pyrex tubes were fused together and served as an insulated handle. The coil was made of the same wire and was mounted inside a porcelain crucible with a 45 mm diameter, which was mounted on a swinging bracket attached to the incubator. A hygrometer was kept in the chamber for control purposes.

Dry materials were introduced through the lock into the glove box. The lock was then evacuated to 0.9 kPa and filled with the gas mixture of the box, whereafter the materials were taken into the box. Using the smaller airlock

the entire process took less than 2 minutes. The larger airlock mostly served as a depository for plates and pipette cassettes. Liquid media and agar plates were introduced in a similar manner except that the lock was evacuated to 19.6 kPa and the evacuation and filling repeated twice. With every entry of materials into the box fresh gas was added to the glove box atmosphere. In this way the composition of the atmosphere in the box could be kept constant. No oxygen analyser was used. Catalyst trays were exchanged and rejuvenated twice a week. Rejuvenation was done by heating the trays to 160 C for 4 hours. Exchange trays were used. Moisture was absorbed by the silica gel, and the gel was dried by heating to 160 C for 4 hours.

To prevent the release of hydrogen sulfide from the cultures, broth were kept in butyl rubber stoppered tubes and plates in plastic bags. Each bag contained a sheet of paper saturated with lead acetate to absorb hydrogen sulfide. The anaerobiosis of the box was verified by the following indicators: alkaline methylene blue (E_0-150 mV), resazurin (E_0-42 mV), indigocarmine (E_0-300 mV) and benzylviologen (E_0-359 mV). The indicators were dissolved in 50 mM potassium phosphate buffer, pH 7.0, to a concentration of 0.1 per cent (wt/vol). Methylene blue indicator was prepared according to Möller (1966).

Bacteriological media

Prereduced media were prepared as described by Holdeman and Moore (1972). Media in plates to be used in the anaerobic glove box were poured outside the box and introduced as soon as they had solidified. Plates were kept in the box for 24 hours for drying and then stored in plastic bags for at least 24 hours before use (Aranki et al. 1969). Horse blood was hemolyzed by freeze--thawing before use in the blood agar plates (Holdeman and Moore, 1972). To some plates palladium black or palladium chloride was added (Aranki et al. 1969; Aranki and Freter, 1972). Mitis salivarius agar contained per litre: 10 g tryptose, 10 g proteose peptone, 1 g dextrose, 50 g sucrose, 4 g dipotassium phosphate, 15 g bacto-agar, 8 ml of a 1 per cent crystal violet solution, and 75 mg trypan-blue. After autoclaving 1 ml of a 1 per cent tellurite solution was added. Rogosa lactobacillus agar contained 75 g Rogosa SL agar per litre. pH was adjusted to 5.4 by the addition of concentrated acetic acid. The medium was boiled for 3 minutes, cooled to 45 C and poured into plates. For isolation of treponemes a prereduced brain heart infusion (BHI) agar was used with the addition of 15 per cent inactivated rabbit serum, 5 µg/ml

cocarboxylase, 800 units/ml nalidixic acid and 800 units/ml polymyxin B (Holdeman and Moore, 1972). A tube with 20 ml of the BHI agar was melted with the stopper on. A filter-sterilized prereduced mixture of serum, cocarboxylase, nalidixic acid, and polymyxin B was added to the BHI agar and the plate was poured. The broth used for isolation of Mycoplasma contained per litre: 21 g mycoplasma broth base, 150 ml inactivated horse serum, 75 ml yeast extract, 75 ml of a 1 per cent thallium acetate solution and 200,000 IE benzyl penicillin. The mycoplasma agar contained per litre: 35 g mycoplasma agar base, 150 ml inactivated horse serum, 75 ml yeast extract, 75 ml of a 1 per cent thallium acetate solution and 300,000 IE benzyl penicillin (Björksten et al.,1975).The yeast extract used in the mycoplasma media was made from bakers yeast according to the method described by Hayflick (1965). For the isolation of yeast a brain heart infusion agar with the addition of penicillin and streptomycin was used. The medium contained per litre: 37 g brain heart infusion broth, and 20 g bacto-agar. After the medium had been autoclaved and cooled to 45 C penicillin and streptomycin were added to the final concentrations of 12 and 40 µg/ml respectively. To demonstrate catalase activity a chocolate agar was used. It contained per litre: 40 g tryptose soy agar, 2 g glucose and 50 ml horse blood. The blood was added after that the medium had been autoclaved and cooled to 75 C. The medium was kept in a water bath at 75 C for 25 minutes, cooled to 45 C and poured into plates. Skimmed milk, prepared from skim milk powder (200 g per litre), was used for the freezing and lyophilization of the bacterial strains. The solution was autoclaved in screw-capped tubes in 5 ml amounts at 115 C for 13 minutes.

Procedure to obtain bacterial samples from the tooth

The procedure outlined by Möller (1966) was followed. The tooth was cleaned with pumice and isolated by a rubber dam. If the tooth had calculus it was scaled and cleaned one week before the sampling. The tooth, the clamp and surrounding parts of the rubber dam was cleansed with 30 per cent hydrogen peroxide for 2 minutes and then swabbed with 5 per cent iodine tincture for 5 minutes. Aseptic technique was used and the operator and assistant wore surgical gloves. After the tincture had dried the tooth surface was swabbed with sterile 5 per cent sodium thiosulphate solution until decoloration of the iodine had occurred. This was to inactivate the iodine tincture before testing the sterility of the tooth surface. A sterile cotton pellet was scrubbed against the lingual surface of the tooth and immediately transferred

to a culture medium and incubated for 3 weeks. From the lingual surface entrance to the pulp chamber was effected first by inverted cone diamond stone and then by steele round burr. Before the pulp chamber was opened a second sterility test sample was taken. Powdered enamel and dentine were removed by a charcoaled paper point and transferred into a culture medium. Chopped meat glucose (CMG) broth, which was prereduced and anaerobically sterilized (PRAS), was used for the samples from the lingual surface and dentin. To avoid oxidation of the PRAS media the sampling tubes were flushed with oxygen-free gas (97 per cent carbon dioxide, 3 per cent hydrogen). In order to facilitate this procedure a "mobile anaerobe laboratory" as described by Fulghum (1971) was used.

When access to the pulp chamber had been gained a small amount of CMG broth was introduced into the canal by a syringe. Care was taken not to overfill the canal. The CMG broth was stored in a tube with combined screw-cap and butyl rubber stopper (Atterberry and Finegold, 1969) from which it was aspirated into the syringe which had previously been flushed with oxygen-free nitrogen gas. If there was exudation from the canal no sampling fluid was used.

The tooth length was known from the isometric roentgenogram. A Hedström file (Maillefer. SA 1338 Ballaiques, Switzerland) was introduced into the canal to the root apex and non-vitality of the pulp was confirmed. Instrumentation and pumping movements with the file were performed as recommended by Möller (1966). A charcoaled paper point was then placed in the canal. Three subsequent points were usually necessary to remove all fluid from the canal. The paper points were quickly transferred to a tube containing 5 ml of CMG broth. A second sample was collected from the canal and transferred in the same way to a PRAS-tube containing 1 ml peptone yeast broth supplemented with inactivated rabbit serum and cocarboxylase (PY-SC) for cultivation of spirochetes (Holdeman and Moore, 1972).

For cultivation of Mycoplasma a third sample was collected and transferred to a screw-capped tube with 2.5 ml of mycoplasma broth. This sample was not protected from oxygen.

Cultivation of the bacterial samples

The samples were then transported to the laboratory. From the broth, which contained the sample, ten-fold serial dilutions in dilution blanks (Holdeman and Moore, 1972) to 10^{-3} were prepared with the V.P.I. Anaerobic culture system (Bellco, Inc., Vineland, N.J., USA). The plates to be incubated aerobically were inoculated. From the original sample and each of the dilutions aliquots of 0.1 ml were spread over the surface of the blood agar plates by means of glass beads. The beads were kept in screw-capped tubes, twenty beads (diam. 5 mm) in each tube. For the cultivation of yeast, 0.1 ml from the original sample in CMG broth was inoculated in the same way on a plate with brain heart infusion agar supplemented with penicillin and streptomycin. A blood agar plate was also triple streaked from the CMG broth. These plates were incubated aerobically for 48 hours at 37 C. All plates used in this study for the initial cultivation of the sample were pre-incubated at 37 C for 48 hours. The control samples from the tooth surface and dentin were incubated at 37 C for 3 weeks. These samples were not cultured on solid media.

The plates to be incubated anaerobically were inoculated in the glove box. From the GMG broth aliquots of 0.1 ml were inoculated on to each of the following media: two blood agar plates, one blood agar plate with palladium, two Mitis salivarius agar plates, two Rogosa lactobacillus agar plates. A blood agar plate was also triple streaked from the CMG broth. From each of the dilutions aliquots of 0.1 ml were inoculated on to each of two blood agar plates and one blood agar plate with palladium. The plates were packed in a plastic bag with a sheet of lead acetate paper to absorbe any hydrogen sulfide produced and were incubated in the box at 37 C. As a precautionary measure, in case accidental oxygen leakage should occur in the glove box, one each of the duplicate plates were placed in an anaerobic jar. The jar was filled inside the box with the plates, a lead acetate paper, palladium catalyst, a methylene blue red/ox indicator and a Gas Pak H_2-CO_2 generator (BBL) and incubated outside the box at 37 C. This jar was opened in the box after 14 days. Plates incubated in the glove box were observed for growth daily. When no growth occurred on the second day another blood agar plate was inoculated from the CMG broth. If no growth occurred this was repeated three times with an interval of one week before the sample was considered negative.

From the PY-SC broth for isolation of treponemes, 0.1 ml was inoculated on to a membrane filter (Millipore 0.22 µm) which was placed on the agar surface of brain heart infusion agar supplemented with rabbit serum, cocarboxylase, polymyxin B and nalidixic acid. The inoculation was repeated on to another plate after 24 hours. The plates were incubated in the glove box at 37 C for 7 days and observed daily for hazy growth in the agar. After 7 days the filter was removed and a plug of agar was cut out and placed on a microscopic slide, crushed with a cover slip and examined for evidence of spirochetes by dark field microscopy.

A mycoplasma agar plate was inoculated with a loop and incubated in a Baird-Tatlok jar at 37 C for 14 days. The jar was filled with 95 per cent nitrogen and 5 per cent carbon dioxide. After 4 days another plate was inoculated from the broth and was also incubated in the jar. The plates were observed for mycoplasma colonies every fourth day. If there was any uncertainity as to whether a colony was a mycoplasma colony it was stained with Dienes staining. This was done by H. Persson at the Department of Virology, University of Umeå.

Isolation of bacterial strains

Different types of colonies were registered after two days on the aerobic and seven days on the anaerobic plates. Smears from the colonies were Gram stained and the frequency of different types of colonies was recorded.

Three colonies of each type were triple streaked on blood agar plates. Subcultures were made until pure cultures were obtained. Strains growing poorly in broth were incubated in the presence of the following additives;bile solution, Tween 80, vitamin K-Hemin, rabbit serum and serum-TEM 4T (Holdeman and Moore, 1972). Addition of 1 per cent hemolyzed horse blood and 2 per cent volatile fatty acid mix (VFA) (Caldwell and Bryant, 1966) were also tested. If any of these additives significantly enhanced the growth compared to the basal medium peptone yeast glucose (PYG) broth it was added to each tube to be inoculated with the strain.

The strains were coded with capital letter(s) indicating the tooth from which it derived.

Identification of bacterial strains

The bacterial strains were characterized by the methods described in the manual of the Virginia Polytechnic Institute and State University, Anaerobe Laboratory, USA (Holdeman and Moore, ed., 1972). Additional tests performed are described in chapter IV where the results of the identification of strains are presented.

Preservation of bacterial strains

The isolated strains were preserved by freezing (-80 C) and lyophilization. The preparatory steps for freezing were performed in the box. Blood agar slants were inoculated and incubated for four days at 37 C. The colonies of each slant were suspended by means of a Pasteur pipette in 2.5 ml of either CMG broth supplemented with 10 per cent glycerol or prereduced skimmed milk. A screw-capped tube with rubber packing (06/1970 Johnsen and Jorgensson, Ltd., London, England) was filled with the suspension from each slant and stored in a freezer (model LTF, Harris manufacturing Co., Inc., No, Billerica, Mass., USA) at -80 C.

To lyophilize the colonies of each slant were suspended in prereduced skimmed milk and 0.5 ml each of two constricted freeze drying ampoules (19/B/1007 Johnsen and Jorgenssen, Ltd., London, England) were filled with the suspension in the anaerobic box. The orifice of each tube was covered with tape, the ampoules were removed from the box and transported to the centrifugal freeze dryer (Speedivac model 5PS, Edwards High Vacuum, Ltd., Manor Royal, Sussex, England). The tape was removed and the ampoules were centrifuged in a reduced atmospheric pressure until the Pirani instrument showed 20 Pa and kept at this pressure for 24 hours. After checking that the pressure had reached 2.66 Pa, nitrogen was introduced into the freeze-drier. The ampoules were then quickly mounted on the multifold and evacuated to 1.33 Pa for 24 hours. The ampoules were subsequently sealed and kept at 4 C. These methods made it possible to preserve all the isolated strains for at least three years.

This chapter presents the general bacteriological findings in relation to the clinical findings.

It has been shown that the outcome of endodontic treatment depends partly on the elimination of bacteria from infected root canals. A higher incidence of periapical destruction after endodontic treatment occurs when bacteria have been isolated from the root canal just before obturation than if no bacteria were found (Zeldow and Ingle, 1963; Engström et al., 1964; Oliet and Sorin, 1969). However, no specific bacteria have been shown to be responsible for the failure of the treatment (Engström et al., 1964). Nor has it been possible to isolate the specific bacteria responsible for the exacerbation of an existing periapical inflammation which occasionally occurs during endodontic therapy (Bartels et al., 1968). Recently Bergenholtz (1974) found bacteria more frequently in samples from teeth with periapical destruction than in those from teeth without destruction.

Results

Thirty-two teeth injured by trauma in 27 patients were studied. In five of the patients two teeth were studied. Three of the patients attended the clinic because of symptoms of acute inflammation from a previously traumatized tooth. Fifteen of the patients were referred to the Department of Endodontics by dentists for treatment and in nine of the patients teeth injured by trauma were detected during routine dental examinations.

Nine-teen of the 32 teeth exhibited radiographic evidence of periapical bone destruction varying from 2 to 10 mm in diameter. No periapical destruction could be seen in the other teeth (Table III:1).

Table III:1

Bacteriological and clinical findings.

Tooth[1] Code	Tooth[2] designation	Age of patient	Periapical area: rarefaction present	Periapical area: size[3]	Time lapse between trauma and sampling	Bacteria present	Number of bacterial strains isolated	Remarks
B	22	23	+	2	6 months	+	1	
C	31	25	+	10	unknown	+	6	
D	41	24	+	2	8 months	+	6	Pain, tenderness, swelling after treatment
E	11	15	+	2	7 months	+	1	
F	21	23	-		6 months	-		
G	32	25	+	5	10 months	+	7	Pain, tenderness, swelling, exudation after treatment
H	31	32	+	6	5 years	+	6	Pain, tenderness, swelling, exudation on treatment
I	11	14	-		3 months	-		
K	31	24	-		8 months	-		
L	11	12	-		4 weeks	-		
M	32	21	+	2	2 months	+	1	
N	41	21	-		3 months	-		
O	11	16	-		10 months	-		
P	31	25	+	8	4 years	+	10	Pain, tenderness, swelling after treatment
R	21	14	+	2	2 months	-		
S	11	15	-		10 months	-		
T	21	28	-		6 years	-		
U	42	47	+	6	15 years	+	1	
V	22	28	-		6 years	-		
X	32	47	+	2	15 years	+	2	
Y	41	20	-		18 months	-		
Z	21	21	-		12 months	-		
AB	41	56	+	5	6 months	+	12	Pain, tenderness, swelling, exudation on treatment
AC	31	60	+	2	3 months	+	4	
BA	32	20	+	6	unknown	+	8	
IN	21	19	+	2	3 months	+	1	
BS	11	41	-		unknown	-		
UJA	31	16	+	4	5 years	+	2	
UJB	41	16	+	10	5 years	+	9	Pain, tenderness, swelling and exudation after treatment
JH	11	15	-		3 months	-		
BN	31	23	+	8	8 years	+	10	Pain, swelling and exudation on treatment
EL	31	29	+	2	unknown	+	1	
							88	

1) In five patients two teeth were sampled. Teeth B and F, D and K, T and V, U and X, UJA and UJB were from these patients.

2) Two-digit system (Fédération Dentaire Internationale)

3) Diameter in millimetre.

The time that had elapsed between the trauma and the bacteriological sampling was known for 28 teeth. In 18 teeth the interval between trauma and sampling was 12 months or less. Periapical destruction was present in 8 of these teeth. In 10 teeth the interval was more than 12 months. Periapical destruction were present in 7 of these teeth (Table III:2). There was no obvious correlation between occurrence of periapical destruction and the time lapse after the trauma.

Table III:2
Occurrence of periapical destruction and the time lapse after trauma.

	Time lapse after trauma	
Periapical destruction	≤12 months	>12 months
Present	8	7
Not present	10	3

Microorganisms were isolated from 18 out of the 19 teeth with periapical destruction, while no microorganisms were found in any teeth without periapical destruction (Table III:1).

In all 88 bacterial strains were isolated. In one root canal as many as twelve strains were found. More strains were found in teeth with greater periapical destruction than in teeth with less destruction (Table III:3).

Table III:3
Size of the periapical destruction and number of isolated bacterial strains from the pulp cavity.

Number of isolated strains	Size of periapical destruction	
	<5 mm	≥5 mm
<6	8	1
≥6	1	8

Seven patients experienced pain in the periapical region of the studied tooth on or after the treatment. Six or more bacterial strains could be isolated from these teeth. From teeth free from symptoms of pain less than six strains could be isolated in nine cases and more than six strains in only two cases (Table III:4).

Table III:4
Composition of the bacterial flora of 18 teeth with periapical destruction, with and without pain in the periapical region.

	Number of teeth	
	Number of strains isolated from the pulp cavities	
Symptom	<6	≥6
Pain	0	7
No pain	9	2

Teeth with acute periapical inflammation also contained a larger number of bacteria than the other teeth. When the samples from the teeth were diluted in ten-fold steps growth occurred on the plates inoculated from the third dilution in seven cases. Six of these seven samples were from teeth with symptoms of acute inflammation.

Discussion

In the present study 88 bacterial strains were isolated from 18 out of 19 teeth with periapical destruction whereas no bacteria could be isolated from teeth without periapical destruction. The main reason for this clear-cut result may be that the method has facilitated the isolation of most of the bacteria present in the teeth and that contaminations were successfully avoided. The method developed by Möller (1966) for isolation of the teeth from the oral environment was successfully used. No bacteria could be cultivated from the samples collected from the lingual surfaces of the teeth or from the powdered dentin collected during the preparation of the entrance to the pulp chamber. Bacteria which occur regularly in saliva such as Mycoplasma (Morton et al., 1951; Razin et al., 1964) facultatively anaerobic lactobacilli (Sundqvist and Carlsson, 1974) or Streptococcus salivarius (Mejàre, 1975)

could not be detected in any sample.

One reasonable interpretation of these results is that periapical osteitis only develops when the pulp of the traumatized tooth has become infected. No prediction can be made about when bacteria will reach the pulp cavity of traumatized teeth. In some cases the pulp cavity may remain aseptic for a long time and in other cases become infected after a short time. In two teeth in the same patient no periapical destruction and no bacteria were found despite an interval of 6 years between trauma and sampling. In some of the teeth studied by Bergenholtz (1974) no bacteria were found two years after trauma.

The present study suggests that the number of bacterial strains dwelling in the pulp cavity influences the size of the periapical destruction and the development of clinical symptoms such as pain, swelling and exudation. In animal experiments it has been shown that a combination of certain bacterial strains is necessary for the development of abscesses and in these infections the bacteria attain pathogenicity by synergism (Macdonald et al., 1954, 1960, 1963; Hampp and Mergenhagen, 1963; Socransky and Gibbons, 1965). In the present study most of the teeth with infected pulp cavities and periapical destruction had no symptoms of acute inflammation. As most of these teeth contained few bacterial strains it is quite possible that the exacerbations of asymptomatic periapical osteitis in humans may depend on the occurrence of a specific combination of bacterial strains in these teeth.

Larger numbers of bacteria were also isolated from teeth with symptoms of acute inflammation than from the other teeth. It is important to identify the isolated bacterial strains to find out whether some bacteria are more common than others in these teeth or whether the development of acute inflammation depends merely on the presence of a large number of bacteria in the pulp cavity.

The success of the bacteriological technique of the present study is illustrated by the fact that more strains of bacteria could be isolated from the teeth than in previous investigations. As many as 8 or more strains were isolated from 5 of the 18 teeth. Möller (1966) with a conventional but elaborate bacteriological technique, Kantz and Henry (1974) with an anaerobic glove box technique and Wittgow and Sabiston (1975) with PRAS roll tube technique

did not isolate more than six strains from any similar teeth. One reason why the present study detected a high number of strains may be that bacteria from infrequently occurring colonies were considered. Kantz and Henry (1974) did not believe colony morphology to be characteristic and therefore isolated all colonies on an initial culture plate with 50 or less colonies. In the present study it was found that colony morphology was a good aid in detecting different bacterial strains, provided that the anaerobic plates were incubated for one week to permit the development of distinguishable colony characteristics. Different colony types were most easily recognized on plates with 100 or less colonies but additional colony types were often found on plates with 100-300 colonies. Three colonies of the same morphology were usually isolated and identified. In all 255 colonies were isolated. In only two cases the colonies were so similar that it was impossible to distinguish initially different strains. With the method used by Kantz and Henry (1974) strains making up a small part of the flora would have been missed. Careful examination of the colony morphology is best done on plates in an anaerobic box. Using the roll tube technique Berg and Nord (1973) could not isolate more than three strains from any root canal. In the present study the culture conditions and the preservation of the isolated strains meant that no strains were lost during the study. In Kantz and Henry's study (1974) 19 per cent of the isolated strains were lost before they were identified.

The high recovery of bacteria and the fact that no strains were lost in the present study increase the probability that the isolated bacteria are representative of the flora in the teeth and that bacteria of special importance for the pathological changes may be revealed.

IV. TAXONOMY OF ISOLATED BACTERIA

In the previous chapter it was concluded that periapical destruction developed in traumatized teeth only if their pulps had become infected. Some of these teeth exhibited symptoms of acute periapical inflammation such as pain and swelling. From these teeth a greater number of bacteria and a more complex bacterial flora were isolated than from teeth without such symptoms. It was suggested that the acute periapical inflammation may not be the result of a heavy bacterial infection only but may also be due to the presence of a specific pathogenic combination of bacteria in the pulp. However, many of the isolated strains did not fit into recognized bacterial species and their position in a system of classification was uncertain. In order to make it possible to compare the bacterial flora isolated from different teeth, the similarities among the isolated strains were evaluated using a numerical taxonomic analysis according to Sneath (1957 a, b).

Material and methods

The numerical taxonomic analysis included 85 of the 88 strains isolated from dental pulps and 45 reference strains (Table IV:1). Three strains were not included because they were found to be Streptococcus mitis (Deibel and Seeley, 1974), when tested as described by Carlsson (1968).

Characters determined and coded are given in Table IV:2. All tests were done three times for the 85 isolated strains and twice for the reference strains. If the results of a test for a strain were not uniform at first the test was repeated twice for the isolated strains and once for the reference strains. The predominant result was used in the numerical analysis. The reliability of the method was evaluated according to Sneath and Johnson (1972) from the results of 20 randomly chosen reference strains and 25 of the isolated strains. The reproducibility of the tests, was high and the average percentage of discrepancies (p) was 1.1% for the reference strains and 1.4% for the isolated strains.

Table IV:1. Reference strains and their sources

Organisms	Strain designation	Source
Actinomyces israelii	ATCC 12102	ATCC[1]
Actinomyces naeslundii	ATCC 12104	ATCC
Actinomyces odontolyticus	NCTC 9935	NCTC[2]
Actinomyces viscosus	ATCC 15987	ATCC
Arachnia propionica	ATCC 14157	ATCC
Arachnia propionica	A 41	G. Bowden, The London Hospital Medical College, Dental School, London
Bacteroides corrodens	VPI 7815	VPI[3]
Bacteroides fragilis s.s. fragilis	NCTC 10584	NCTC
Bacteroides melaninogenicus s.s. intermedius	NCTC 9336	NCTC
Bacteroides melaninogenicus s.s. melaninogenicus	ATCC 25845	ATCC
Bacteroides melaninogenicus s.s. asaccharolyticus	VPI 4198 (ATCC 25260)	VPI
Bacteroides ochraceus	VPI 9775 (ATCC 27872)	VPI
Bacteroides oralis	ATCC 15930	ATCC
Bacteroides ruminicola s.s. ruminicola	VPI 0051-b (ATCC 19189)	VPI
Bifidobacterium bifidum	NCTC 10471	NCTC
Bifidobacterium bifidum	NCTC 10472	NCTC
Bifidobacterium dentium	VPI 7511 (ATCC 27534)	VPI
Bifidobacterium dentium	L 109d-3	S. Edwardsson, University of Lund School of Dentistry, Malmö, Sweden
Campylobacter bubulus	NCTC 10355	NCTC
Campylobacter fetus	NCTC 10354	NCTC
Clostridium ramosum	ATCC 25554	ATCC
Clostridium perfingens type A	NCTC 8237	ATCC
Eubacterium alactolyticum	ATCC 19301	ATCC
Eubacterium lentum	ATCC 25559	ATCC
Eubacterium limosum	ATCC 8486	ATCC
Fusobacterium gonidiaformans	ATCC 25563	ATCC
Fusobacterium naviforme	ATCC 25832	ATCC
Fusobacterium necrophorum	NCTC 10575	NCTC
Fusobacterium nucleatum	NCTC 10562	NCTC
Fusobacterium russi	ATCC 25533	ATCC
Fusobacterium varium	ATCC 8501	ATCC
Lactobacillus catenaforme	ATCC 25536	ATCC
Leptotrichia buccalis	NCTC 10249	NCTC
Peptococcus aerogenes[4]	ATCC 14963	ATCC
Peptococcus prevotii	ATCC 9321	ATCC
Peptococcus saccharolyticus	ATCC 14953	ATCC
Peptococcus variabilis	ATCC 14955	ATCC
Peptococcus variabilis[5]	ATCC 14956	ATCC
Peptostreptococcus anaerobius	VPI 4330-1 (ATCC 27337)	VPI
Propionibacterium avidum	ATCC 25577	ATCC
Propionibacterium acidi-propionici	ATCC 25562	ATCC
Propionibacterium acne	NCTC 737	NCTC
Streptococcus constellatus	ATCC 27823	ATCC
Streptococcus intermedius	ATCC 27335	ATCC
Streptococcus morbillorum	ATCC 27824	ATCC

1) ATCC = American Type Culture Collection 12301 Park Lawn, Drive Rockville, Maryland, USA.

2) NCTC = National Collection of Type Cultures. Central Public Health Laboratory, Colindale Avenue, London.

3) VPI = Virginia Polytechnic Institute and State University Blacksburg, Virginia, USA.

4) According to Holdeman and Moore (1972) a Peptococcus asaccharolyticus strain.

5) According to Holdeman and Moore (1972) a Peptococcus magnus strain.

Particulars about the tests (Table IV:2).

Tests 1, 2, 3, 4, 5, 7, 8, 9. Cell morphology, arrangement of the cells, and motility were studied by phase contrast microscopy in wet mounts from peptone yeast extract glucose (PYG) broth. The size of the cells was determined using an eyepiece micrometer. The cells were Gram stained as described by Holdeman and Moore (1972). Scanning electron microscopy (S.E.M.) was used if the cell morphology could not be determined by light microscopy. If the strain did not grow in the broth, material from colonies on blood agar were used for the S.E.M. The specimen was treated as described by Elmros et al. (1975).

Test 6. The ability to form spores was tested by growing the strains on chopped meat agar slants (Holdeman and Moore, 1972) at 30 C. After 7 days a starch broth (Holdeman and Moore, 1972) was inoculated from the slant. The starch broth was heated to 80 C for 10 minutes in a heating block (Grant Instruments, Cambridge, England) and then incubated at 37 C. To detect any living cells in the starch broth samples from the broth were inoculated on blood agar after 1 and 7 days. The blood agar was incubated under anaerobic conditions at 37 C for 7 days. This procedure was repeated after the chopped meat agar slant had been incubated for 21 days. Clostridium ramosum (ATCC 25554) was used as positive control. No strains isolated from dental pulps formed spores.

Tests 10, 11, 12, 13. Colonial morphology and pigmentation (Lamanna and Malette, 1965) were studied on blood agar after the plates had been in anaerobic incubation for 7 days. Pigment production was also studied on blood agar incubated for 3 days at 37 C in air supplemented with 30 per cent carbon dioxide.

Tests 14, 15. The ability to grow in air or in air supplemented with 30 per cent carbon dioxide was studied with blood agar plates incubated for 3 days at 37 C. The plates were inoculated as described for the antibiotic sensitivity tests (tests 86-95).

Tests 16-41. The terminal pH in broth supplemented with carbohydrate (Holdeman and Moore, 1972) was recorded electrometrically (Beckman Zeromatic pH meter, Beckman instruments Ltd., England) after the broth had been incubated for 14 days.

Tests 42, 43, 45. The hydrolysis of esculin, starch and gelatin was tested as described by Holdeman and Moore (1972).

Test 44. Hydrolysis of casein was recorded when a clear zone occurred around streaks on brain heart infusion agar (Holdeman and Moore (1972) supplemented with 25 g per litre skim milk powder (Oxoid L 31) after 7 days incubation.

Test 46. Hydrolysis of egg protein was recorded when a clear zone occurred around streaks on egg yolk agar (Holdeman and Moore, 1972) after 7 days incubation.

Test 47. Catalase activity was tested by adding a solution containing 5 per cent hydrogen peroxide to colonies on egg yolk agar and chocolate agar and observing for the formation of gas bubbles. The plates had been incubated under anaerobic condition at 37 C for 7 days and thereafter been exposed to air for at least 30 min before hydrogen peroxide was added.

Test 48. Deoxyribonuclease activity was studied on DNAse agar (Oxoid CM 321). After incubation at 37 C for 7 days the plate was flooded with 1 N HCl and inspected for areas of clearing around the streaks.

Tests 49-58 were performed as described by Holdeman and Moore (1972).

Tests 59-68. PYG-broth was supplemented with the following substances: Tween 80, vitamin K-hemin, TEM 4T/rabbit serum, rabbit serum, bile or pyruvate in concentrations recommended by Holdeman and Moore (1972); laked horse blood was added to a final concentration of 1 per cent (vol/vol) and vitamin fatty acid mix (Caldwell and Bryant, 1966) to 2 per cent (vol/vol); sodium formate and sodium fumarate were added to a final concentration of 100 mM and 120 mM, respectively (Kafkewitz, 1975). The pH and turbidity at 500 nm (Beckman DB-G Grating Spectro-Photometer, Beckamn instruments Ltd., England) were measured after incubation for 14 days at 37 C. An additive was considered to stimulate growth if the turbidity of the broth with additive was twice that of PYG broth or if the pH of the broth was 0.5 units lower than in PYG broth. An additive was considered to inhibit growth if the turbidity of the broth with the additive was less than half the turbidity of PYG broth or the pH was 0.5 units higher than in PYG broth.

Tests 69-85. Fermentation products in the base (PY) broth and in supplements of the broth - with glucose, pyruvic acid, lactic acid or threonine - were analyzed by gas chromatography as described by Yamada and Carlsson (1975). The presence of formic acid was not determined. No internal standard was used.

Tests 86-95. The following antibiotics were used; kanamycin sulphate (AB Ferrosan,Malmö, Sweden), neomycin sulphate (Upjohn Co., Kalamazoo Mich., USA), penicillin G sodium (AB Kabi, Stockholm, Sweden), erythromycin lactobionate (Abott Laboratories, Chicago, Ill., USA), tetracycline chlorid (ACO Läkemedel AB, Solna, Sweden), rifampicin (AB Ferrosan, Malmö, Sweden), Polymyxin B sulphate (Novo industri A/S, Bagsvaerd, Denmark), bacitracin and colistin sodium methansulfonat (Lundbeck & Co A/S, Copenhagen, Denmark), metronidazol (AB Leo, Helsingborg, Sweden).

Erythromycin was dissolved in ethanol. Rifampicin was dissolved by mixing 300 mg of the antibiotics with 5 mg sodium formaldehydsulfoxylate (Rongalite) and 4 ml sterile water and adjusting the pH to 8.3 with 0.1N NaOH. The other antibiotics were dissolved in sterile water. All solutions were diluted in sterile water. They were not sterilized. PDM Antibiotic Sensitivity Medium (AB Biodisk, Solna, Sweden) supplemented with 5 per cent (vol/vol) defibrinated horse blood was used in the "agar dilution" method (Washington and Barry, 1974). The agar plates were prepared in air, introduced into the anaerobic box as soon as they had solidified and allowed to dry overnight before use. The concentrations of the antibiotics are expressed as micrograms of active substance per ml of medium. Tenfold dilution steps were used with the following ranges; kanamycin (10,000 - 0.01), neomycin (1,000 - 0.01), penicillin G (0.1 - 0.0001), erythromycin (1 - 0.0001), tetracycline (1 - 0.0001), rifampicin (10 - 0.0001), polymyxin B (1,000 - 0.1), bacitracin (100 - 0.0001), colistin (1,000 - 0.01) and metronidazol (10 - 0.0001). The strain to be tested was streaked on a blood agar slant and incubated at 37 C for 4 days. The colonies on the slant were suspended in 5 ml PYG broth giving a concentration of 10^6 - 10^7 organisms/ml. The suspensions were introduced into tubes (6 by 25 mm) in an apparatus similar to that used by Steers et al. (1959) for inoculation of the agar plates. In the present apparatus capillary tubes ("Microcaps", Drummond Scientific Co, Broomall, Pa, USA) were used instead of solid rods (Steers et al., 1959). The capillary tubes delivered 1/µl (0.8 - 1.2/µl) of the suspension on a spot of 3 to 5 mm on the agar surface.
Sixty capillary tubes were used and 60 strains could be tested at the same time on a plate with a diameter of 9 cm. The inoculum which contained 10^3 - 10^4 organisms was allowed to dry on the surface before the plates were incubated at 37 C. Plates free from antibiotics were inoculated before and after each series of plates with antibiotics was inoculated to check that viable organisms were present throughout the procedure. After 4 days the lowest concentration of antibiotics which inhibited growth was recorded.

For the numerical taxonomic analysis 95 characteristics were used. Characteristic which did not vary among the strains were excluded. Multi-stage characteristic 1, 2, 3, 4, 8, 9, 10, 11, 16 - 41, 58, 84, 86 - 95 were coded according to Beers and Lockhart (1962). The number of differences (nd) was determined for each pair of strains (Sneath, 1957 b). For clustering the unweighted pair-group average linkage method was used (Sokal and Michener, 1958). The

Table IV:2

Characteristics of bacterial groups in the dendrogram (Diagram 1).
Figures in the table denote number of strains fullfilling the listed criteria.

Characters determined and encoded for numerical analysis		FUSOBACTERIUM 1	BACTERIODES 1	PEPTOSTREPTOCOCCUS	EUBACTERIUM 1	EUBACTERIUM 2	VEILLONELLA	PEPTOCOCCUS 1	PEPTOCOCCUS 2	EUBACTERIUM 3-4	FUSOBACTERIUM 2	VIBRIO, CAMPYLOBACTER	PROPIONIBACTERIUM	LACTOBACILLUS 1	LACTOBACILLUS 2	LACTOBACILLUS 3	STREPTOCOCCUS	BIFIDOBACTERIUM	ACTINOMYCES 1	ACTINOMYCES 2	ARACHNIA	SELENOMONAS	BACTEROIDES 2	BACTEROIDES 3	BACTEROIDES 4	BACTEROIDES 5
		No of strains																								
		18	3	6	3	4	2	5	7	8	3	9	2	5	2	5	3	4	4	5	4	2	2	2	6	3
CELL MORPHOLOGY																										
1. Length	<1 µm	-	-	-	-	-	-	5	5	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-
	1-3 µm	5	2	6	1	-	2	-	2	8	3	4	-	3	-	5	1	1	1	1	1	1	-	-	-	-
	>3 µm	13	-	-	2	4	-	-	-	-	-	5	-	-	1	-	-	3	3	3	1	1	-	-	-	3
	variable length (<75% cells in any one of above categories)	-	1	-	-	-	-	-	-	-	-	-	2	2	1	-	-	-	-	1	2	-	2	2	6	-
2. Width	filamentous, length to width ratio of 20:1	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
	slender, length to width 3:1	4	-	-	2	4	-	-	-	-	1	9	-	-	2	1	-	4	4	3	1	2	-	-	-	-
	short, length to width 2-3:1	5	1	-	1	-	-	-	-	7	2	-	-	2	-	1	-	-	-	1	-	-	-	-	-	-
	oval, length to width 1:1	-	1	6	-	-	2	5	7	-	-	-	-	-	-	3	3	-	-	-	-	-	-	-	-	-
	variable width (<75% in any of above categories)	-	1	-	-	-	-	-	-	1	-	-	2	3	-	-	-	-	-	1	3	-	2	2	6	-
3. Shape of cell	straight rod	16	1	-	2	-	-	-	-	7	3	6	-	1	-	1	-	-	-	-	-	-	-	1	-	2
	curved rod	-	-	-	-	3	-	-	-	1	-	1	-	-	2	-	-	-	-	2	-	2	-	-	-	1
	spiral rod	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	cocco-bacillary	1	1	-	1	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-
	coccus (spherical)	-	-	6	-	-	2	5	7	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-
	pleomorphic (<75% in any of above categories)	1	1	-	-	1	-	-	-	-	-	-	2	4	-	1	-	4	4	3	4	-	2	1	6	-
4. Round ended cell		10	3	6	3	4	2	5	7	8	3	9	2	5	2	5	3	4	4	5	4	2	2	2	6	-
Tapered cell		8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
5. Branching		-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	4	4	3	4	-	-	-	-	-
6. Spores present		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7. Motility		-	-	-	-	-	-	-	-	-	-	8	-	-	-	-	-	-	-	-	-	2	-	-	-	-
8. Gram stain reaction	negative	18	3	-	-	-	2	2	-	-	3	9	-	-	-	-	-	-	-	-	-	2	2	2	6	3
	positive	-	-	6	3	4	-	-	7	8	-	-	2	5	2	5	3	4	4	5	4	-	-	-	-	-
	variable (<75% in either of above categories)	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9. Cell arrangement	single cells	17	2	-	1	3	-	-	-	4	3	9	-	-	-	-	-	-	-	-	-	2	-	-	-	3
	chains of cells, short (<5 cells/chain)	-	-	-	2	-	-	4	7	4	-	-	-	1	-	3	2	-	-	-	-	-	-	-	-	-
	chains of cells, long (>5 cells/chain)	-	-	6	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
	clumping of coccal forms	-	-	-	-	-	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	variable cell arrangement (<75% in any of above categories)	1	1	-	-	1	-	-	-	-	-	-	2	4	2	2	-	4	4	5	4	-	2	2	6	-
COLONY MORPHOLOGY																										
10. Size	<1 mm	-	1	-	-	-	-	5	1	2	3	2	-	2	-	-	-	-	-	-	-	-	-	-	-	-
	1-3 mm	11	2	5	3	4	2	-	6	6	-	2	2	3	1	5	3	4	4	5	4	2	2	2	6	3
	>3 mm	7	-	1	-	-	-	-	-	-	-	5	-	-	1	-	-	-	-	-	-	-	-	-	-	-
11. Shape	effuse	3	-	-	-	-	1	-	-	-	-	7	-	2	-	-	-	-	-	-	-	-	-	-	-	-
	raised	-	-	1	-	-	-	3	-	6	-	2	-	3	-	5	-	-	-	-	-	-	-	-	-	-
	convex	9	2	2	-	1	1	2	7	2	3	-	1	-	-	-	1	2	2	4	-	1	1	2	1	3
	umbonate	1	-	1	3	-	-	-	-	-	-	-	-	-	1	-	1	2	-	1	-	1	-	-	2	-
	pulvinate	1	1	2	-	3	-	-	-	-	-	-	1	-	1	-	1	-	2	-	4	-	1	-	3	-
	convex rugose	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12. Colonies red when grown in CO_2		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	3	-	-	-	-	-	-
13. Colonies black when grown in anaerobic condition		-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	6	-
OXYGEN TOLERANCE																										
14. Growth in air		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	3	2	3	-	-	-	-	-
15. Growth in an atmosphere of 30% CO_2 in air		-	-	1	-	-	-	-	-	-	-	2	2	-	-	-	3	2	4	5	4	-	-	-	-	2
ACID PRODUCED IN BROTH CONTAINING																										
16. Arabinose	≤5.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	2	-	-	-	-	-	-
	5.6-5.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	1	-	-	-
	>5.8	18	3	6	3	4	2	5	7	8	3	9	2	5	2	4	3	1	3	3	4	2	1	2	6	3
17. Cellobiose	≤5.5	-	-	-	-	-	-	-	-	-	-	-	-	1	2	2	1	3	2	-	-	-	-	2	-	1
	5.6-5.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	2	-	-	2
	>5.8	18	3	6	3	4	2	5	7	8	3	9	2	4	-	3	2	1	-	5	4	2	-	-	6	-
18. Erythritol	≤5.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	5.6-5.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	>5.8	18	3	6	3	4	2	5	7	8	3	9	2	5	2	5	3	4	4	5	4	2	2	2	6	3
19. Esculin	≤5.5	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	2	-	-	-	-	-	-	-
	5.6-5.8	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	2	-	-	-	-	-	1	1	-	2
	>5.8	18	3	6	3	4	2	5	7	8	3	9	2	4	1	4	1	4	2	5	4	2	1	1	6	1

Table IV:2 continued

Characters determined and encoded for numerical analysis		FUSOBACTERIUM 1	BACTEROIDES 1	PEPTOSTREPTOCOCCUS	EUBACTERIUM 1	EUBACTERIUM 2	VEILLONELLA	PEPTOCOCCUS 1	PEPTOCOCCUS 2	EUBACTERIUM 3-4	FUSOBACTERIUM 2	VIBRIO, CAMPYLOBACTER	PROPIONIBACTERIUM	LACTOBACILLUS 1	LACTOBACILLUS 2	LACTOBACILLUS 3	STREPTOCOCCUS	BIFIDOBACTERIUM	ACTINOMYCES 1	ACTINOMYCES 2	ARACHNIA	SELENOMONAS	BACTEROIDES 2	BACTEROIDES 3	BACTEROIDES 4	BACTEROIDES 5
		No of strains																								
		18	3	6	3	4	2	5	7	8	3	9	2	5	2	5	3	4	4	5	4	2	2	2	6	3
ACID PRODUCED IN BROTH CONTAINING																										
20. Fructose	≤5.5	-	-	3	-	3	-	-	-	-	-	-	2	4	2	5	3	4	4	4	4	2	1	2	3	3
	5.6-5.8	7	-	3	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	2	-
	>5.8	11	3	-	3	-	2	5	7	8	3	9	-	1	-	-	-	-	-	-	-	-	-	-	1	-
21. Glucose	≤5.0	-	-	-	-	-	-	-	-	-	-	-	-	-	2	5	2	4	3	1	-	1	1	-	2	-
	5.0-5.5	2	-	1	-	1	-	-	-	-	-	-	2	5	-	-	1	-	1	4	4	1	1	2	3	3
	5.6-5.8	6	1	2	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
	>5.8	10	2	3	3	-	2	5	7	8	3	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22. Inositol	≤5.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-
	5.6-5.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
	>5.8	18	3	6	3	4	2	5	7	8	3	9	2	5	2	5	3	4	-	5	4	2	2	2	6	3
23. Lactose	≤5.5	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	1	4	4	4	2	2	2	2	-	3
	5.6-5.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-
	>5.8	18	3	6	3	4	2	5	7	8	3	9	2	5	-	5	2	-	-	-	1	-	-	-	6	-
24. Maltose	≤5.5	-	-	-	-	-	-	-	-	-	-	-	-	3	-	5	3	4	4	2	4	2	1	2	3	3
	5.6-5.8	1	-	2	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	1	-	-	1	-	3	-
	>5.8	17	3	4	3	4	2	5	7	8	3	9	2	1	1	-	-	-	-	2	-	-	-	-	-	-
25. Mannitol	≤5.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	1	-	4	1	-	-	-	-
	5.6-5.8	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	>5.8	18	3	6	3	-	2	5	7	8	3	9	2	5	2	5	3	2	3	5	-	1	2	2	6	3
26. Mannose	≤5.5	-	-	-	-	-	-	-	-	-	-	-	1	-	2	5	3	3	4	-	3	-	2	2	3	3
	5.6-5.8	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1	-	-	-	1	-	-	-	-
	>5.8	17	3	6	3	4	2	5	7	8	3	9	-	5	-	-	-	-	-	5	1	1	-	-	3	-
27. Melezitose	≤5.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	1	-	-	-	-	-	-	-
	5.6-5.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	2	-	-	-	-	-	-	-
	>5.8	18	3	6	3	4	2	5	7	8	3	9	2	5	2	5	3	-	1	5	4	2	2	2	6	3
28. Melibiose	≤5.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	4	-	-	2	-	1	-	-
	5.6-5.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-
	>5.8	18	3	6	3	4	2	5	7	8	3	9	2	5	2	5	3	-	-	5	4	-	-	1	6	3
29. Raffinose	≤5.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	4	-	4	2	2	2	2	3
	5.6-5.8	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	2	-
	>5.8	17	3	6	3	4	2	5	7	8	3	9	2	5	2	5	3	-	-	4	-	-	-	-	2	-
30. Rhamnose	≤5.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	5.6-5.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	1	-	2	-	1	1
	>5.8	18	3	6	3	4	2	5	7	8	3	9	2	5	2	5	3	4	1	5	3	2	-	2	5	2
31. Ribose	≤5.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	2	2	-	-	-	-	-	-
	5.6-5.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
	>5.8	18	3	6	3	4	2	5	7	8	3	9	2	5	2	5	3	-	1	3	4	2	2	2	6	3
32. Salicin	≤5.5	-	-	-	-	-	-	-	-	-	-	-	-	1	2	1	2	3	3	-	-	-	1	-	-	-
	5.6-5.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
	>5.8	18	3	6	3	4	2	5	7	8	3	9	2	4	-	4	1	1	-	5	4	2	1	2	6	3
33. Sorbitol	≤5.5	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	1	-	-	-	-
	5.6-5.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1	-	-
	>5.8	18	3	6	3	3	2	5	7	8	3	9	2	5	2	5	3	4	3	5	1	1	2	1	6	3
34. Starch (soluble)	≤5.5	-	-	-	-	-	-	-	-	-	-	-	-	2	2	-	-	3	1	1	-	-	2	1	4	3
	5.6-5.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	1	-	-	-	-	2	-
	>5.8	18	3	6	3	4	2	5	7	8	3	9	2	3	-	5	2	1	2	3	4	2	-	1	-	-
35. Sucrose	≤5.5	-	-	-	-	-	-	-	-	-	-	-	-	4	2	5	3	4	4	4	3	1	2	2	4	3
	5.6-5.8	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1	-
	>5.8	17	3	6	3	4	2	5	7	8	3	9	2	1	-	-	-	-	-	1	-	1	-	-	1	-
36. Trehalose	≤5.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	2	3	3	-	3	-	-	-	-	-
	5.6-5.8	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1	-	-
	>5.8	18	3	6	3	4	2	5	7	8	3	9	2	5	1	2	1	1	1	5	1	2	2	1	6	3
37. Xylose	≤5.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	3	2	3	-	-	-	-	-	-
	5.6-5.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	1	-	1	-	-	-	-	-
	>5.8	18	3	6	3	4	2	5	7	8	3	9	2	5	2	2	2	1	1	2	3	2	2	2	6	3
38. Dulcitol	≤5.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	5.6-5.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
	>5.8	18	3	6	3	4	2	5	7	8	3	9	2	5	2	5	3	4	4	5	4	2	2	1	6	3
39. Glycerol	≤5.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	5.6-5.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	2	1	-	-	-	-	-
	>5.8	18	3	6	3	4	2	5	7	8	3	9	2	5	2	5	3	4	3	3	3	2	2	2	6	3
40. Inulin	≤5.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	3	-	-	-	2	2	3	3
	5.6-5.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-
	>5.8	18	3	6	3	4	2	5	7	8	3	9	2	5	2	5	3	3	1	5	4	2	-	-	1	-
41. Sorbose	≤5.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	5.6-5.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
	>5.8	18	3	6	3	4	2	5	7	8	3	9	2	5	2	5	3	4	4	5	4	2	2	2	5	3

Table IV:2 continued

Characters determined and encoded for numerical analysis	FUSOBACTERIUM 1	BACTEROIDES 1	PEPTOSTREPTOCOCCUS	EUBACTERIUM 1	EUBACTERIUM 2	VEILLONELLA	PEPTOCOCCUS 1	PEPTOCOCCUS 2	EUBACTERIUM 3-4	FUSOBACTERIUM 2	VIBRIO, CAMPYLOBACTER	PROPIONIBACTERIUM	LACTOBACILLUS 1	LACTOBACILLUS 2	LACTOBACILLUS 3	STREPTOCOCCUS	BIFIDOBACTERIUM	ACTINOMYCES 1	ACTINOMYCES 2	ARACHNIA	SELENOMONAS	BACTEROIDES 2	BACTEROIDES 3	BACTEROIDES 4	BACTEROIDES 5
	No of strains																								
	18	3	6	3	4	2	5	7	8	3	9	2	5	2	5	3	4	4	5	4	2	2	2	6	3
BIOCHEMICAL TESTS																									
42. Hydrolysis of esculin	1	-	-	-	-	-	-	-	-	-	-	-	1	2	5	2	4	4	1	1	1	2	2	-	3
43. Hydrolysis of starch	-	-	-	-	-	-	-	-	-	-	-	-	1	2	-	-	2	-	-	-	-	2	1	6	-
44. Hydrolysis of casein	1	3	-	-	-	-	-	-	-	-	1	2	-	2	-	1	3	3	5	3	-	-	2	6	2
45. Hydrolysis of gelatin	1	3	-	-	-	-	-	-	-	-	1	2	-	-	-	-	-	-	-	-	-	2	2	6	-
46. Hydrolysis of egg protein	2	1	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1	2	6	-
47. Catalase present	-	-	-	-	-	1	-	-	-	-	1	2	-	-	-	-	-	1	1	-	-	-	-	-	-
48. DNA:se present	10	3	2	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	3	-	1	2	2	6	1
49. Lecithinase present	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-
50. Lipase present	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	2	5	-
51. Ammonia produced in AMC broth	17	2	5	2	-	-	-	4	-	2	-	1	-	-	-	-	-	-	-	-	-	1	-	4	1
52. Ammonia produced in L-arginine broth	17	2	6	2	-	2	-	4	4	3	-	2	5	-	3	2	-	3	-	1	-	-	1	2	1
53. Acetoin produced	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	1	5	4	1	-	-	-	3
54. Hydrogen sulfide produced	16	3	6	3	-	-	-	-	-	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-
55. Indol produced	15	3	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	6	-
56. Nitrate reduced	-	-	-	-	-	2	-	-	3	-	9	2	-	-	-	-	-	4	3	4	2	-	-	-	1
57. Nitrite reduced	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
58. No gas produced	8	2	-	3	-	-	5	7	8	3	9	2	5	2	5	3	3	4	5	4	-	2	2	6	3
Small amount of gas produced	6	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	2	-	-	-	-
Large amount of gas produced	4	-	6	-	4	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GROWTH STIMULATED BY																									
59. Tween 80	-	-	-	-	-	-	-	-	-	-	-	1	5	-	5	-	-	-	-	-	-	1	1	1	-
60. Vitamin K-hemin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-
61. TEM and rabbit serum	2	-	1	-	-	-	-	-	-	-	-	-	2	-	5	-	-	-	-	-	-	1	2	3	-
62. Vitamin fatty acid solution (VFA)	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
63. Horse blood	-	-	1	-	-	-	-	-	-	-	-	1	-	-	2	-	-	-	-	1	-	-	1	2	-
64. Rabbit serum	1	-	1	-	-	-	-	-	-	-	-	-	1	-	2	-	-	-	-	1	-	-	2	2	-
65. Formate and fumarate	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
66. Pyruvate	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GROWTH INHIBITED BY																									
67. Bile	17	3	6	3	4	-	-	-	-	3	9	2	5	2	5	3	4	4	5	4	2	2	2	6	3
68. Formate and fumarate	18	2	6	2	4	-	-	3	-	2	-	1	2	2	2	3	-	3	5	4	1	2	2	6	3
PRODUCTS FROM GLUCOSE																									
69. Ethanol	1	1	1	-	2	-	-	1	-	-	-	-	4	2	5	1	3	-	-	-	-	-	-	-	-
70. Acetic acid	18	3	6	3	4	2	-	7	2	1	-	2	5	2	5	3	4	4	5	4	2	2	2	6	3
71. Propionic acid	10	3	6	-	-	2	5	-	-	-	-	2	-	-	-	-	-	-	-	4	2	-	-	-	-
72. Iso-butyric acid	-	3	6	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-
73. Butyric acid	18	3	6	-	4	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
74. Iso-valeric acid	-	3	6	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	6	-
75. Valeric acid	-	-	3	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
76. Iso-hexanoic acid	-	-	6	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
77. Hexanoic acid	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
78. Octanoic acid	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
79. Lactic acid	8	-	4	2	-	-	-	1	1	-	-	-	5	2	5	3	4	3	-	-	1	-	-	-	-
80. Pyruvic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
81. Succinic acid	-	1	-	-	1	-	-	-	-	-	9	-	-	-	-	1	-	4	5	4	-	2	2	6	3
82. Lactic acid to acetic acid ratio more than 10:1	-	-	-	-	-	-	-	-	-	-	-	-	2	-	4	3	-	-	-	-	-	-	-	-	-
83. Less iso-butyric, iso-valeric, iso-hexanoic acids produced from glucose broth (PYG) than from base broth (PY)	-	3	6	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	6	-

Table IV:2 continued

Characters determined and encoded for numerical analysis		FUSOBACTERIUM 1	BACTEROIDES 1	PEPTOSTERPTOCOCCUS	EUBACTERIUM 1	EUBACTERIUM 2	VEILLONELLA	PEPTOCOCCUS 1	PEPTOCOCCUS 2	EUBACTERIUM 3-4	FUSOBACTERIUM 2	VIBRIO, CAMPYLOBACTER	PROPIONIBACTERIUM	LACTOBACILLUS 1	LACTOBACILLUS 2	LACTOBACILLUS 3	STREPTOCOCCUS	BIFIDOBACTERIUM	ACTINOMYCES 1	ACTINOMYCES 2	ARACHNIA	SELENOMONAS	BACTEROIDES 2	BACTEROIDES 3	BACTEROIDES 4	BACTEROIDES 5
		No of strains																								
		18	3	6	3	4	2	5	7	8	3	9	2	5	2	5	3	4	4	5	4	2	2	2	6	3
84. PRODUCTS FROM LACTIC ACID																										
Acetic and propionic acid		1	-	-	-	-	2	-	1	-	-	-	2	-	-	-	-	-	-	-	3	2	-	-	-	-
Acetic, butyric or hexanoic acid		14	1	4	-	4	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ethanol and acetic acid		-	-	1	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Succinic acid		-	-	-	-	-	-	-	-	-	-	9	-	-	-	-	-	-	3	5	-	-	2	2	6	3
Lactic acid not utilized		3	2	1	3	-	-	5	4	8	2	-	-	5	2	5	3	4	1	-	1	-	-	-	-	-
PRODUCTS FROM THREONINE																										
85. Propionic acid		15	-	6	1	-	2	4	-	-	-	-	2	-	-	-	-	-	-	-	4	2	-	-	-	2
ANTIBIOTIC SENSITIVITY	µg/ml																									
86. Sensitive to kanamycin	100	17	0	0	3	0	2	5	7	8	3	9	2	0	0	5	2	3	4	5	3	2	0	0	0	0
87. Sensitive to neomycin	100	13	0	1	2	0	1	5	5	7	3	9	2	1	0	5	2	4	3	2	2	2	0	0	4	0
88. Sensitive to penicillin G	0.01	14	1	4	3	4	2	4	1	3	3	5	2	4	2	0	3	0	3	5	2	1	1	2	6	0
89. Sensitive to erythromycin	0.1	5	3	6	3	4	0	2	1	8	3	8	2	5	1	5	3	4	4	5	3	1	0	0	6	3
90. Sensitive to tetracycline	0.1	17	2	6	3	1	2	4	5	3	2	8	1	1	1	2	1	0	2	4	3	0	0	2	6	2
91. Sensitive to rifampicin	0.1	13	3	6	3	4	1	0	7	8	3	0	2	0	0	5	3	1	4	5	4	2	2	2	6	3
92. Sensitive to polymyxin B	100	18	3	1	0	0	2	5	0	4	3	9	2	0	0	0	0	2	0	1	0	2	2	0	6	1
93. Sensitive to bacitracin	1	5	3	6	1	4	0	2	1	3	1	0	2	3	2	2	0	4	4	5	3	1	0	0	1	0
94. Sensitive to colistin	100	17	1	0	0	0	2	5	0	1	0	9	1	0	0	0	0	1	0	0	0	2	2	0	6	0
95. Sensitive to metronidazol	0.1	18	3	6	3	4	2	2	7	8	3	8	0	4	0	0	0	0	0	0	0	2	1	1	6	0

admission of any strain to a cluster was based on the arithmetic mean of the nd-values for that strain with the members of the cluster. The relationship between two clusters was calculated as the arithmetic mean of nd-values among the strains in the two clusters. The calculations were carried out on a CDC 3300 computer at Umeå Data Centre (UMDAC).

Cell wall analysis was performed on Gram positive rods by Dr. G.H. Bowden, Dental Bacteriology Laboratory, The London Hospital Medical College, Dental School, London E 1, England. Cell walls were examined for lysine, ornithine, diaminopimelic acid (DL. DAP and LL. DAP), galactose, glucose, mannose, rhamnose and 6-deoxytalose using the methods described by Edwardsson and Bowden (1974). The results of this analysis were not included in the numerical taxonomic analysis.

Results

A. Numerical taxonomy

The summarized result of the numerical evaluation of the affinities among the isolated strains and the reference strains is presented in Diagram IV:1. No predetermined similarity level was followed when this heterogenous collection of bacteria was divided into phenons.

Most of the isolated strains clustered with one or more of the reference strains and phenons, including reference strains, were designated with the genus names of the reference strains. The other phenons in which no reference strains were included were designated after considering the characteristics of the included strains. The phenons were divided into groups when the isolated strains clustered with reference strains of different species or subspecies within the genus and when analysis of the characteristics of the strains made it clear that groups of strains which were different were present within the phenon. Some of the reference strains were not included in these groups. Such strains were Eubacterium limosum (ATCC 8486), Peptococcus prevotii (ATCC 9321), P. aerogenes (ATCC 14963), P. variabilis (ATCC 14955, 14956), Propionibacterium avidum (ATCC 25577), Bacteroides ruminicola (VPI 0051-b) and Bacteroides fragilis (NCTC 10584). Other reference strains had a very low similarity with any of the isolated strains and were not included in any phenon. Peptococcus saccharolyticus (ATCC 14953), Clostridium ramosum (ATCC 25554), Leptotrichia buccalis (NCTC 10249), Clostridium perfringens (NCTC 8237) and Propionibacterium acidici-propionici (ATCC 25562) were such strains. These reference strains are not included in the summarized characterisation of the groups given in Table IV:2 and in Table IV:3 where the inter- and intragroup affinities among the groups are given.

A dendrogram is the result of a polytethic classification. In monothetic classification the assignement of bacteria into genera and species is largely based on some commonly accepted characteristics such as reaction to the Gram stain and the shape of the cells. These characteristics made up only 4 out of 95 used in this numerical analysis. The fact that Gram positive and negative strains and cocci and rods were not randomly mixed in the phenons suggests that these two characteristics are associated with others of the bacteria. As many as 26 of the characteristics were fermenting tests and this resulted in the clustering of the fermentative strains in the one part of the dendrogram and the non- or weak-fermentative strains in the other part and in general at a higher similarity level.

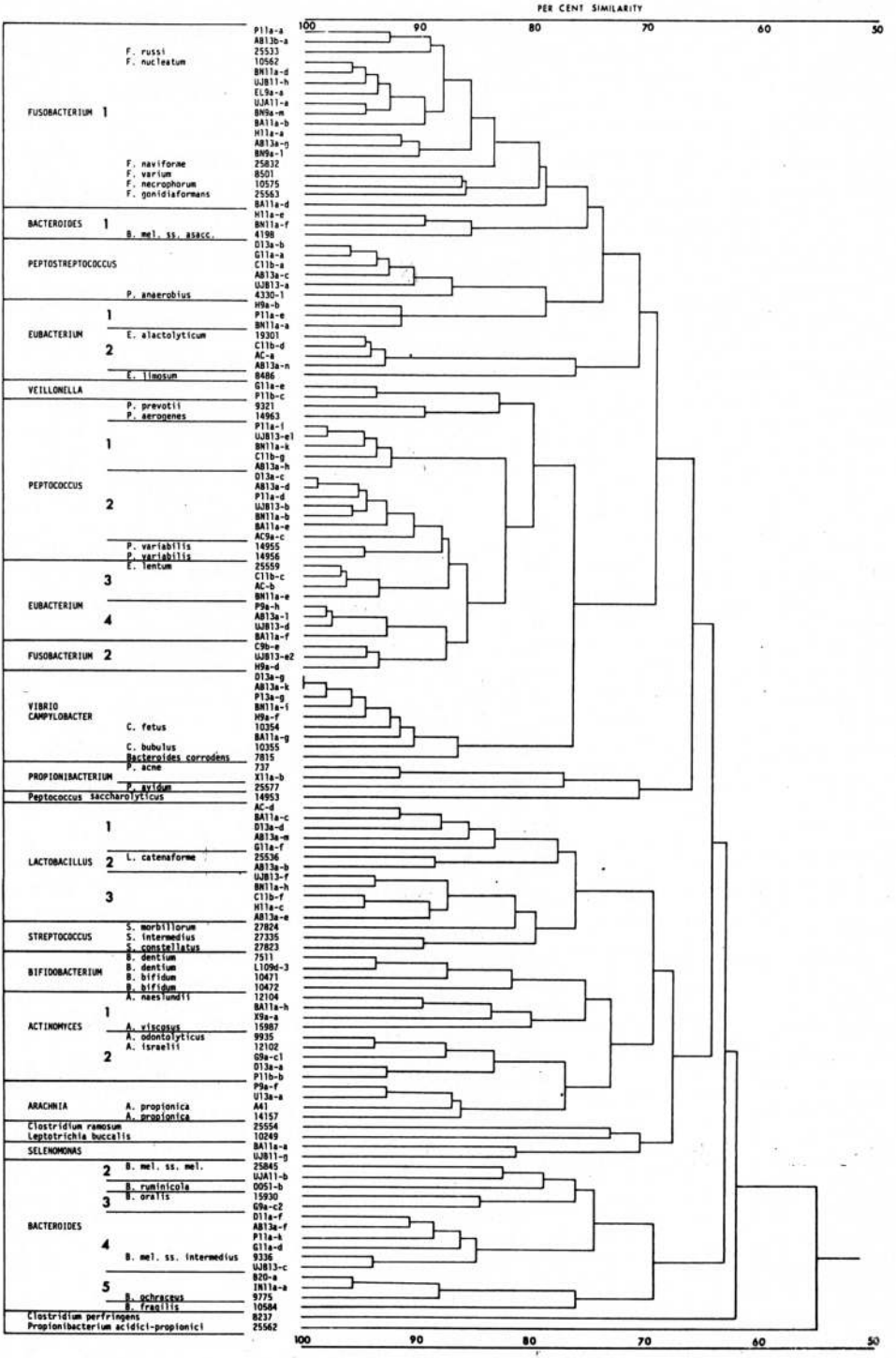

Diagram IV:1. Dendrogram illustrating the phenetic resemblance among the 130 strains studied. The identity of the reference strains are given in Table IV:1.

Table IV:3. Mean percentage similarity values within and between the groups in the numerical taxonomic analysis.

	FUSOBACTERIUM 1	BACTEROIDES 1	PEPTOSTREPTOCOCCUS	EUBACTERIUM 1	EUBACTERIUM 2	VEILLONELLA	PEPTOCOCCUS 1	PEPTOCOCCUS 2	EUBACTERIUM 3, 4	FUSOBACTERIUM 2	VIBRIO/CAMPYLOBACTER	PROPIONIBACTERIUM	LACTOBACILLUS 1	LACTOBACILLUS 2	LACTOBACILLUS 3	STREPTOCOCCUS	BIFIDOBACTERIUM	ACTINOMYCES 1	ACTINOMYCES 2	ARACHNIA	SELENOMONAS	BACTEROIDES 2	BACTEROIDES 3	BACTEROIDES 4	BACTEROIDES 5
FUSOBACTERIUM 1	85																								
BACTEROIDES 1	76	87																							
PEPTOSTREPTOCOCCUS	74	78	91																						
EUBACTERIUM 1	75	74	82	92																					
EUBACTERIUM 2	74	70	76	77	94																				
VEILLONELLA	77	70	74	73	73	95																			
PEPTOCOCCUS 1	74	69	69	72	71	82	94																		
PEPTOCOCCUS 2	77	72	76	81	80	82	84	94																	
EUBACTERIUM 3, 4	76	73	73	81	80	79	82	87	91																
FUSOBACTERIUM 2	81	76	73	79	79	77	81	85	87	94															
VIBRIO/CAMPYLOBACTER	74	66	63	74	72	76	81	77	80	80	92														
PROPIONIBACTERIUM	65	68	64	65	66	70	66	68	70	67	61	82													
LACTOBACILLUS 1	67	64	70	72	75	69	70	77	78	75	67	67	86												
LACTOBACILLUS 2	62	60	63	67	73	61	65	70	70	66	62	66	76	89											
LACTOBACILLUS 3	63	60	66	67	69	65	68	76	75	69	63	65	79	74	89										
STREPTOCOCCUS	63	60	68	69	70	67	68	77	74	69	62	67	75	76	79	84									
BIFIDOBACTERIUM	54	52	54	59	63	55	57	64	63	59	56	63	68	75	71	69	85								
ACTINOMYCES 1	53	49	53	57	62	55	54	62	62	59	58	63	64	69	67	71	75	82							
ACTINOMYCES 2	64	63	63	67	72	65	64	72	72	68	67	70	70	70	67	72	71	73	86						
ARACHNIA	60	60	62	63	69	66	63	67	68	63	61	73	68	67	66	71	69	74	78	88					
SELENOMONAS	69	63	63	65	71	73	71	70	71	70	72	65	65	65	65	65	66	64	66	70	81				
BACTEROIDES 2	59	60	55	57	61	59	58	63	62	60	59	60	64	67	62	64	62	64	64	61	64	82			
BACTEROIDES 3	58	59	53	56	60	57	56	63	61	60	57	61	65	67	65	65	60	64	66	65	60	77	84		
BACTEROIDES 4	63	72	62	63	62	62	59	63	63	61	61	63	63	62	59	61	54	54	65	60	57	76	73	87	
BACTEROIDES 5	66	60	59	63	68	62	62	68	68	68	65	63	66	71	66	71	60	71	74	73	69	73	75	63	90

B. Identification of the isolated strains

The grouping derived from the numerical taxonomic analysis is followed when the strains are identified. The strains are described in the following order:

For identification of strains the criteria suggested in Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974) are followed.

FUSOBACTERIUM group 1.

This group consists of 12 isolated strains and the reference strains F. russi (ATCC 25533), F. nucleatum (NCTC 10562), F. naviforme (ATCC 25832), F. varium (ATCC 8501), F. necrophorum (NCTC 10575) and F. gonidiaformans (ATCC 25563.

Morphology. The strains were Gram negative, non-sporulating rods. They were nonmotile and obligately anaerobic. Cells were usually long and thin (0.5 by 5-20 µm) with tapered ends, but some strains had short cells (0,5 by 1.5-2 µm) with rounded ends (Table IV:4).

Colonies. Three types of colony morhology could be recognized on anaerobic blood agar incubated for 5 days. Five strains grew with flat to low convex colonies 3 to 5 mm in diameter, circular gray-white, translucent with a mottled appearance. Nine strains had convex to pulvinate, circular to slightly irregular, translucent, gray colonies, 2 to 3 mm in diameter. Four strains had circular to irregular, lobate, white colonies, 2 mm in diameter and with a convoluted surface giving the colonies a "molar-tooth" or "raspberry"-like appearance.

Cultural characteristics.PY-glucose broth cultures had a flocculent or granular appearance and the pH was 5.5-6.0 after incubation for 14 days. Two of the strains (BA11a-b, BA11a-d) were stimulated by TEM-rabbit serum.

Biochemical reactions. The characteristics of the strains are given in Table IV:4. No strains fermented arabinose, cellobiose, dulcitol, erythritol, esculin, glycerol, inositol, inulin, lactose, mannitol, melezitose, melibiose, rhamnose, ribose, salicin, sorbitol, sorbose, starch, trehalose, xylose or hydrolyzed starch. Catalase was not produced by any strain. Nitrate was not reduced by any strain.

Fermentation products. In PY-glucose broth all strains produced acetic and butyric acid (Table IV:4). The amount of acetic and butyric acid varied from 2 to 20 mM and the proportions from 1:20 to 7:1. Ten strains produced propionic acid (2-10 mM) and eight strains lactic acid (15-20 mM). One strain produced ethanol. Fifteen strains produced propionic acid from threonine. In PY-lactate broth one strain produced propionic acid.

Antibiotic sensitivity.

	Number of strains							
	Minimum inhibitory concentration (µg/ml)							
	1000	100	10	1	0.1	0.01	0.001	0.0001
Kanamycin	1	6	8	3				
Neomycin	5	8	3	2				
Penicillin G				2	2	2	10	2
Erythromycin[1)]				2	1	2	1	1
Tetracycline			1		14	3		
Rifampicin		1	1	2	7	2		4
Polymyxin B		2	2	6	8			
Bacitracin	1	11	1		2	1	2	
Colistin	1	3		3	9	2		
Metronidazol					1	3	3	11

1) 11 strains grew at the highest used concentraition (1 µg/ml)

Eleven of the strains clustered with F. russi (ATCC 25533) and F. nucleatum (NCTC 10562). Six of the strains (BN11a-d, UJB11-h, E19a-a, UJA11-a,BN9a-m, BA11a-b) have a high similarity with F. nucleatum and are assigned to that species. None of the other strains could be identified at species level. One strain (BA11a-d) differs in many respects from the others. It hydrolyzed esculin and produced acid from raffinose and sucrose and the antibiotic sensibility pattern was different. The strains not assigned to any species are designated Fusobacterium group 1.

Table IV:4

Characteristics of strains assigned to *Fusobacterium* groups.

	FUSOBACTERIUM group 1									group 2
	F. russi	*F. nucleatum*	*F. naviforme*	*F. varium*	*F. necrophorum*	*F. gonidia-formans*				
	ATCC 25533	NCTC 10562	ATCC 25832	ATCC 8501	NCTC 10575	ATCC 25563	H11a-a AB13a-g	BA11a-d 9 strains[3]		C9b-e UJB13-e2 H9a-d
Products from PYG broth[1]	a, b	a,p,B,L	a,B,l	a,P, B,l	A,p,B	a,B	a,b	A,p, b,e	a(6),A(3),p(6), b(4),B(5),L(5)	a(1),b
Lactate → propionate	-	-	-	-	+	-	-	-	-	-
Threonine → propionate	+	+	-	+	+	+	-	+	+	-
Acid produced from[2]										
Fructose	-	w	-	w	-	-	-	-	w(5)	-
Glucose	-	w	a	a	-	w	-	w	w(3)	-
Maltose	-	-	-	-	-	w	-	-	-	-
Mannose	-	-	-	w	-	-	-	-	-	-
Raffinose	-	-	-	-	-	-	-	w	-	-
Sucrose	-	-	-	-	-	-	-	w	-	-
Esculin hydrolyzed	-	-	-	-	-	-	-	+	-	-
Casein hydrolyzed	-	-	-	-	+	-	-	-	-	-
Gelatin hydrolyzed	-	-	-	-	-	+	-	-	-	-
Protein hydrolyzed	-	-	-	-	+	-	-	-	+(1)	-
DNAse present	-	-	+	-	+	+	-	-	+(7)	-
Lipase present	-	-	-	-	+	-	-	-	-	-
Ammonia produced	+	+	+	+	+	+	+(1)	+	+	+(2)
Hydrogen sulfide produced	+	+	+	+	+	+	-	+	+	-
Acetoin produced	-	-	-	-	-	-	-	-	+(4)	-
Indol produced	-	+	+	+	+	+	-	+	+	-
Gas produced	+	-	-	+	+	+	-	+	+(5)	-
Short cells, rounded ends	+	-	-	+	+	+	+(1)	-	+(2)	+

1) Products in PYG broth: capital letters indicate ≥ 10 mM; small letters < 10 mM; A, acetic acid; B, butyric acid; E, ethanol; P, propionic acid; L, lactic acid.

2) a, pH 5.5 or below; w, pH 5.6-5.8; -, pH above 5.8

3) Numbers in parentheses denote numbers of strains fulfilling the listed criteria.
+, positive reaction; -, negative reaction.

FUSOBACTERIUM group 2

This group consists of three isolated strains.

Morphology. The strains were Gram negative rods, non-sporulating, nonmotile and obligately anaerobic. Cells were 0.5 by 1.5 µm with rounded ends.

Colonies. On blood agar incubated 5 days the colonies were convex, small gray pin-points.

Cultural characteristics. The strains grew poorly in broth and were not stimulated by any additive.

Biochemical reactions. The characteristics of the strains are given in Table IV:4. None of the tested carbohydrates was fermented. Two of the strains (C9b-e, UJB13-e2) produced ammonia in AMC broth and three strains in L-arginine broth (Table IV:2).

Fermentation products. In PY-glucose broth the strains produced a small amount of butyric acid (2-5 mM) and one strain produced acetic acid. No propionic acid was produced in PY-threonine broth or in PY-lactate broth.

Antibiotic sensitivity.

	Minimum inhibitory concentration (µg/ml)		
	C9b-e	UJB13-e2	H9a-d
Kanamycin	10	1	0.1
Neomycin	100	10	10
Penicillin G	0.001	0.001	0.0001
Erythromycin	0.1	0.1	0.1
Tetracycline	0.1	1	0.01
Rifampicin	0.0001	0.0001	0.0001
Polymyxin B	100	100	100
Bacitracin	10	1	100
Colistin	1 000	1 000	1 000
Metronidazol	0.0001	0.01	0.0001

These strains have few distinguishing characteristics and can only be described as Gram negative rods producing butyric acid. They cannot be assigned to any species. They are therefore designated Fusobacterium group 2.

BACTEROIDES group 1.

This group consists of two isolated strains and the reference strain B. melaninogenicus ss. asaccharolyticus (VPI 4198).

Morphology. The strains were Gram negative, non-sporulating, nonmotile and obligately anaerobic. The shape of the cells was cocco/bacillary, 0.75 by 1-1.25 µm.

Colonies. On blood agar incubated under anaerobic conditions for 7 days the colonies of the isolated strains were 1 mm in diameter, circular, entire and convex. They became black after 5-7 days. The reference strain grew with pulvinate colonies 2 to 3 mm in diameter.

Cultural characteristics. The strains grew only on blood agar and often lost their viability after 5-7 days. Poor growth occurred in Py-glucose broth and the final pH was 6.4 for the isolated strains and 5.6 for the reference strain. No additive stimulated growth in broth.

Biochemical reactions. The characteristics of the strains are given in Table IV:5. No carbohydrates were fermented. Gelatin and casein were digested. DNAse was present. Lipase and lecitinase were not detected.

Fermentation products. In PY broth the isolated strains produced acetic (8 mM), propionic (1.5 mM), isobutyric (1 mM), butyric (4-12 mM) and isovaleric (4-8 mM) acid. The average proportions were 10:2:1:8:4. The reference strain produced higher amounts of propionic acid (10-15 mM) and also succinic acid (5-10 mM). In PY-glucose broth the same acids were produced in smaller amounts. No strain produced propionic from threonine.

Antibiotic sensitivity.

	Minimum inhibitory concentration (µg/ml)		
	VPI 4198	H11a-e	BN11a-f
Kanamycin	>10 000	>10 000	10 000
Neomycin	>1 000	1 000	1 000
Penicillin G	0.1	0.1	0.0001
Erythromycin	0.01	0.001	0.001
Tetracycline	1	0.1	0.1
Rifampicin	0.01	0.01	0.0001
Polymyxin B	100	100	100
Bacitracin	1	1	1
Colistin	1 000	100	1 000
Metronidazol	0.001	0.0001	0.01

From the numerical taxonomic analysis it was concluded that the two isolated strains could be identified as Bacteroides melaninogenicus ss. asaccharolyticus. There was a low similarity between the group of B. melaninogenicus ss. asaccharolyticus (Bacteroides 1) and the other groups of Bacteroides (Bacteroides 2 and 4) in which subspecies of B. melaninogenicus were found. The similarity to the Bacteroides group 2 (B. mel. ss. melaninogenicus) was 60 per cent and with Bacteroides group 4 (B. mel. ss. intermedius) 72 per cent. Williams et al. (1975) have recently shown that there are differences among the subspecies of B. melaninogenicus in the mean base composition of DNA, in cell wall composition, and in the malate dehydrogenases. The taxonomic status of these subspecies has to be reconsidered.

BACTEROIDES group 2

This group consists of one isolated strain and the reference strain Bacteroides melaninogenicus ss. melaninogenicus. (ATCC 25845)

Morphology. The strains in this group were Gram negative, non-sporulating, non-motile and obligately anaerobic rods. Cells were 1.0 by 1.0 to 10 µm. Rods and coccoid forms occurred in the same culture.

Colonies. After anaerobic incubation for 7 days colonies on blood agar were 2 mm in diameter, circular, entire, convex to pulvinate, smooth, translucent and gray. In the center of the colony of the isolated strain a brown-red area could sometimes be seen after incubation for more than a week. The reference strain grew with unpigmented colonies.

Cultural characteristics. The strains did not survive on blood agar for more than 7-10 days under anaerobic conditions. The strains grew well in PYG broth and the final pH was 5.2-5.5. The growth of the isolated strain was enhanced by the addition of Tween 80 and TEM-serum.

Biochemical reactions. The characteristics are given in Table IV:5. DNAse was present in both strains and lipase and proteinase in the isolated strain. Erythritol, glycerol, inositol, mannitol, melezitose, ribose and xylose were not fermented.

Fermentation products. In PY-glucose broth acetic acid (10-15 mM) and succinic acid (10-12 mM) were produced. The reference strain also produced isovaleric acid (3-5 mM).

Antibiotic sensitivity.

	Minimum inhibitory concentration (µg/ml)	
	ATCC 25845	UJA11-b
Kanamycin	10 000	10 000
Neomycin	1 000	1 000
Penicillin G	0.1	0.01
Erythromycin	1	1
Tetracycline	1	1
Rifampicin	0.001	0.0001
Polymyxin B	10	100
Bacitracin	10	10
Colistin	10	100
Metronidazol	0.001	1

BACTEROIDES group 3

This group consists of one isolated strain and the reference strain Bacteroides oralis (ATCC 15930).

Morphology. The strains were Gram negative, non-sporulating, nonmotile and obligately anaerobic rods. The shape of the cells varied and both cocco--bacillary and long forms were present. The size was 1.0 by 1.5-10 µm.

Colonies. On blood agar incubated anaerobically for 5 days the isolated strain grew with colonies which were 2 mm, circular, entire, convex, smooth, white--yellow and sometimes with a red pigmented centre. The reference strain grew with black pigmented colonies.

Cultural characteristics. After incubation for 14 days in PY-glucose broth the pH was 5.0-5.4. Growth of both strains was stimulated by the addition of rabbit serum, serum - TEM.

Biochemical reactions. The characteristics are summarized in Table IV:5. DNAse, lipase and proteinase were produced. Erythritol, glycerol, inositol, mannitol, melezitose, ribose and xylose were not fermented.

Fermentation products. In PY-glucose broth acetic and succinic acids were produced in the proportion 1:5. Propionic acid was not produced from threonine.

Antibiotic sensitivity.

	Minimum inhibitory concentration (µg/ml)	
	ATCC 15930	G9a-c2
Kanamycin	10 000	10 000
Neomycin	1 000	1 000
Penicillin G	0.001	0.01
Erythromycin	1	1
Tetracycline	0.1	0.1
Rifampicin	0.01	0.0001
Polymyxin B	1 000	1 000
Bacitracin	100	100
Colistin	1 000	>1 000
Metronidazol	1	0.01

The two isolated strains in groups 2 and 3 of Bacteroides (UJA11-b, G9a-c2) show great similarity to B. oralis. They produce on blood agar colonies which occasionally are faintly red-brown pigmented but never black. Black pigmented colonies appears to be the sole basis for differentiation between B. oralis and B. mel. ss. melaninogenicus (Holdeman and Moore, 1972, 1974), but in the present study the B. oralis, ATCC 15930, produced black colonies and the B. mel. ss. melaninogenicus, ATCC 25845, did not produce black colonies. Holbrook and Duerden (1974) have also noticed the black colonies of B. oralis, ATCC 15930, and have proposed reclassification of the strain as a strain of B. melaninogenicus. Obviously the ability to produce pigment is not a good basis for differation of the two species. The uncertain taxonomic position of the reference strains makes it preferable not to assign either of the two isolated strains to any of the species. They are therefore only identified as Bacteroides species.

Table IV:5

Characteristics of strains assigned to Bacteroides groups.

Characteristics	BACTEROIDES group 1		BACTEROIDES group 2		BACTEROIDES group 3		BACTEROIDES group 4		BACTEROIDES group 5	
	B. mel. ss. asacch.		B. mel. ss. mel.		B. oralis		B. mel. ss. interm.		B. ochraceus	
	VPI 4198	H11a-e[3] BN11a-f	ATCC 25845	UJA11-b	ATCC 15930	G9a-c2	NCTC 9336	D11a-f AB13a-f P11a-k G11a-d UJB13-c	VPI 9775	B20-a IN11a-a
Black pigment prod.	+	-	-	-	+	-	+	+	-	-
Products from PYG broth[1]	A,P,B, ib,iv,s	a,p,b, ib,iv	A,S,iv	A,S	a,S	a,S	A,S,ib,iv	A,iv,S(5) ib(2)	a,S	A,S
Threonine → propionate	-	-	-	-	-	-	-	-	-	+
Acid produced from[2]										
Arabinose	-	-	w	-	-	-	-	-	-	-
Cellobiose	-	-	w	w	a	a	-	-	a	w
Dulcitol	-	-	-	-	w	-	-	-	-	-
Esculin	-	-	-	w	-	w	-	-	w	w(1)
Fructose	-	-	a	w	a	a	a	a(2),w(2)	a	a
Glucose	w	-	a	a	a	a	a	a(4),w(1)	a	a
Inulin	-	-	a	a	a	a	a	a(2),w(2)	a	a
Lactose	-	-	a	a	a	a	-	-	a	a
Maltose	-	-	a	w	a	a	a	a(2),w(3)	a	a
Mannose	-	-	a	a	a	a	a	a(2)	a	a
Melibiose	-	-	w	w	-	a	-	-	-	-
Raffinose	-	-	a	a	a	a	a	a(1),w(2)	a	a
Rhamnose	-	-	w	w	-	-	w	-	w	-
Salicin	-	-	-	a	-	-	-	-	-	-
Sorbitol	-	-	-	-	w	-	-	-	-	-
Sorbose	-	-	-	-	-	-	w	-	-	-
Starch	-	-	a	a	-	a	a	a(3),w(2)	a	a
Sucrose	-	-	a	a	a	a	a	a(3),w(1)	a	a
Trehalose	-	-	-	-	w	-	-	-	-	-
Gelatine hydrolyzed	+	+	+	+	+	+	+	+	-	-
Esculin hydrolyzed	-	-	+	+	+	+	-	-	+	+
Starch hydrolyzed	-	-	+	+	-	+	+	+	-	-
Nitrate reduced	-	-	-	-	-	-	-	-	-	+(1)
Indol produced	+	+	-	-	-	-	+	+	-	-
Ammonia produced in AMC broth	+	+(1)	+	-	-	-	+	+(3)	+	-
Hydrogen sulfide produced	+	+	-	-	-	-	-	-	-	-
Acetoin produced	-	-	-	-	-	-	-	-	+	+
Gas produced	+	-	-	-	-	-	-	-	-	-

1) Products from PYG broth: capital letters indicate ≥ 10 mM; small letters <10 mM; A, acetic acid; B, butyric acid; ib, isobutyric acid; iv, isovaleric acid; P, propionic acid; S, succinic acid.

2) a, pH in broth 5.5 or below; w, pH 5.6-5.8; -, pH above 5.8; +, positive reaction; -, negative reaction.

3) Numbers in parentheses denote number of strains fulfilling the listed criteria.

BACTEROIDES group 4

This group consists of five isolated strains and the reference strain Bacteroides melaninogenicus ss. intermedius (NCTC 9336).

Morphology. The strains in this group were Gram negative non-sporulating, nonmotile and obligately anaerobic rods. Cells varied in size and both cocco--bacillary and long forms were present (1.0 by 1.5-10 µ).

Colonies. On blood agar incubated anaerobically for 7 days the colonies were 2 to 3 mm, circular, entire, convex to pulvinate and black or dark brown.

Cultural characteristics. After incubation for 14 days the pH in PY-glucose broth varied among the strains (4.8-5.8). The growth of some strains in broth was stimulated by Tween 80 (1), Vitamin K-hemin (2), TEM serum (3), blood and serum (2).

Biochemical reactions. The characteristics are given in Table IV:5. All strains hydrolyzed protein and produced DNAse. Lipase was produced by the isolated strains. Erythritol, glycerol, inositol, mannitol, melezitose, ribose, xylose were not fermented.

Fermentation products. In PY-glucose broth acetic, isovaleric and succinic acids were produced by all strains and isobutyric acid by three strains. The proportions between acetic, isobutyric, isovaleric and succinic acids were 2: 1:1:10. The same acids were produced in PY broth but in greater quantities. No propionic acid was produced from threonine or lactate.

Antibiotic sensitivity.

	Minimum inhibitory concentration (µg/ml)					
	NCTC 9336	D11a-f	G11a-d	P11a-k	AB13a-f	UJB13-c
Kanamycin	10 000	10 000	10 000	1 000	>10 000	10 000
Neomycin	1 000	100	100	100	1 000	100
Penicillin G	0.01	0.01	0.01	0.01	0,01	0,01
Erythromycin	0.01	0.01	0.01	0.01	0.01	0.01
Tetracycline	0.1	0.01	0.1	0.01	0.1	0.1
Rifampicin	0.01	0.0001	0.01	0.001	0.0001	0.001
Polymyxin B	1	1	1	0.1	10	1
Bacitracin	1	10	10	10	10	10
Colistin	1	1	1	1	1	1
Metronidazol	0.001	0.0001	0.0001	0.0001	0.1	0.01

The isolated strains are similar to the reference strain NCTC 9336 and are identified as B. melaninogenicus ss. intermedius.

BACTEROIDES group 5

This group consists of two isolated strains and the reference strain Bacteroides ochraceus (VPI 9775).

Morphology. The strains were Gram negative rods, non-sporulating nonmotile and anaerobic. Cells had tapered ends and were long and thin, usually 0.75 by 5-20 µm. Filaments of 100 µm length could sometimes be seen.

Colonies. On blood agar incubated anaerobically for 7 days the colonies were 2 mm, circular, entire, convex, shiny, translucent and yellow to light orange. Rhizoid colonies with growth penetrating into the agar occurred.

Cultural characteristics. Final pH in PY-glucose broth was 5.0-5.4. Growth was not significally enhanced by any of the additives. The isolated strains but not the reference strain grew as surface colonies on blood agar incubated in air supplemented with 30 per cent carbon dioxide.

Biochemical reactions. The characteristics are given in Table IV:5. Casein was digested by one of the isolated strains and by the reference strain. Lipase and lecitinase were not produced. DNAse was produced by the reference strain. Erythritol, glycerol, inositol, mannitol, melezitose, ribose, xylose were not fermented.

Fermentation products. In PY-glucose broth acetic and succinic acid were produced in proportions 1:2. Propionic acid was produced from threonine by the isolated strains.

Antibiotic sensitivity.

	Minimum inhibitory concentration (µg/ml)		
	VPI 9775	B20-a	IN11a-a
Kanamycin	1 000	1 000	1 000
Neomycin	1 000	1 000	1 000
Penicillin G	0.1	0.1	0.1
Erythromycin	0.1	0.1	0.1
Tetracycline	0.1	0.1	1
Rifampicin	0.1	0.0001	0.0001
Polymyxin B	100	1 000	1 000
Bacitracin	100	100	100
Colistin	1 000	1 000	1 000
Metronidazol	1	10	10

The isolated strains are identified as Bacteroides ochraceus.

SELENOMONAS

This group consists of two isolated strains.

Morphology. The strains were Gram negative, motile, non-sporulating, anaerobic, curved rods. At primary isolation cells were helical and in chains but at subculture single curved cells 0.5 by 2-6 μm with rounded ends occurred. SEM showed flagella on the concave side of the cells.

Colonies. After incubation for 7 days colonies were 2 to 3 mm in diameter, circular, entire, convex to umbonate. The colonies of one strain were gray and the centers mottled, the other strain had red-yellow colonies.

Cultural characteristics. In PY-glucose broth the final pH was 4.5 for one strain and 5.2 for the other strain. Growth was not stimulated by any of the additives. Bile inhibited the growth of both strains.

Biochemical reactions. Both strains fermented fructose, glucose, lactose, maltose, melibiose, raffinose. One strain (UJB11-g) also fermented mannitol, mannose, sorbitol and sucrose. None of the strains fermented arabinose, cellobiose, dulcitol, erythritol, esculin, glycerol, inositol, inulin, melezitose, rhamnose, ribose, salicin, sorbose, starch, trehalose or xylose. Both strains reduced nitrate and produced small amounts of gas. One strain (UJB11-g) hydrolyzed esculin and produced acetoin. Starch, casein, egg protein and gelatine were not hydrolyzed. Indol, ammonia, hydrogen sulfide were not produced. Lipase, lecitinase and catalase were not present. DNAse was present in one strain.

Fermentation products. In PY-glucose broth acetic acid (15-40 mM) and propionic acid (35-60 mM) were produced by both strains. One strain (UJB11-g) produced lactic acid (40 mM). From pyruvate, lactate and threonine acetic and propionic acids were produced.

Antibiotic sensitivity.

	Minimum inhibitory concentration (µg/ml)	
	BA11a-a	UJB11-g
Kanamycin	1	0.1
Neomycin	10	1
Penicillin G	0.1	0.01
Erythromycin	1	0.1
Tetracycline	1	1
Rifampicin	0.01	0.1
Polymyxin B	1	100
Bacitracin	1	10
Colistin	0.1	100
Metronidazol	0.001	0.1

No reference strain of Selenomonas was available but both strains conform well to the description of that genus. The two species S. sputigena and S. ruminatum are similar in many respects but differ in the fermentation of cellobiose, salicin and dulcitol, production of hydrogen sulfide, G+C content of DNA, ecology (Bryant, 1974) and growth in bile (Holdeman and Moore, 1972). The two isolated strains are identified as S. sputigena.

This group consists of six isolated strains and the reference strains Bacteroides corrodens (VPI 7815), Campylobacter fetus (NCTC 10354) and Campylobacter bubulus (NCTC 10355).

Morphology. The six isolated strains were Gram negative, motile, non-sporulating, obligately anaerobic rods. Cells had round ends and were 0.5 by 2-6 µm in size. One strain (BAlla-g) had curved cells, usually with one or two coils per cell, the other strains were straight rods. The cells had a polar flagellum, but few were motile.

Colonies. On blood agar incubated anaerobically for 7 days five of the isolated strains grew with colonies 3 to 5 mm in diameter, irregular with undulating margins, effuse and translucent. These colonies had thin spreading edges and produced slight zones of depression in the agar around the colonies. One strain (BAlla-g) grew with colonies 1 mm in diameter, circular, entire, raised to umbonate, translucent and gray. Of the reference strains C. fetus and C. bubulus grew with small pin pointed, circular, raised, translucent colonies. B. corrodens grew with colonies which were translucent, irregular and effuse with spreading edges and which produced zones of depression in the agar around the colonies.

Cultural characteristics. There was no visible growth in PY-glucose broth. Addition of formate and fumarate produced visible turbidity in the broth. None of the other additives stimulated growth. The pH of the broth did not change when growth was stimulated by addition of formate and fumarate. None of the isolated strains grew on the surface of blood agar incubated aerobically or in air supplemented with 30 per cent carbon dioxide.

Biochemical reactions. Carbohydrates were not fermented. Nitrate was reduced by all strains and nitrite by two of the strains. Hydrogen sulfide was produced by all the isolated strains. None of the isolated strains hydrolyzed esculin, starch, casein, egg protein and gelatin or produced indol, catalase, ammonia, acetoin or gas. DNAse, lipase and lecithinase could not be detected.

Fermentation products. No fermentation products could be detected in PY-glucose broth. When PY-glucose broth was supplemented with formate and fumarate large amounts of succinic acid (125-175 mM) were produced by the isolated strains, B. corrodens (VPI 7815) and C. bubulus (NCTC 10355). Succinic acid was also produced from pyruvate and lactate. One strain (BAlla-g) produced acetoin from pyruvate.

Antibiotic sensitivity.

	Minimum inhibitory concentration (µg/ml)								
	VPI 7815	NCTC 10354	NCTC 10355	D13a-g	H9a-f	P13a-g	AB13a-k	BA11a-g	BN11a-i
Kanamycin	0.1	1	1	1	0.1	0.1	0.1	0.1	0.1
Neomycin	1	1	10	1	1	1	1	1	1
Penicillin G	0.001	1	0.01	0.01	0.1	0.01	0.01	0.1	0.1
Erythromycin	0.1	0.1	0.1	0.1	0.1	0.1	0.1	1	0.1
Tetracycline	0.01	0.1	0.1	0.1	0.1	0.01	0.1	1	0.01
Rifampicin	1	>10	>10	1	10	1	1	>10	1
Polymyxin B	0.1	10	0.1	0.1	10	10	10	10	0.1
Bacitracin	1 000	100	10	1 000	1 000	1 000	1 000	1 000	1 000
Colistin	0.01	10	0.1	100	1	100	100	10	0.1
Metronidazol	1	0.01	0.01	0.01	0.01	0.1	0.1	0.01	0.01

The isolated strains clustered in the numerical taxonomic analysis with the reference strains Bacteroides corrodens (VPI 7815), Campylobacter fetus (NCTC 10354) and Campylobacter bubulus (NCTC 10355). B. corrodens was nonmotile, hydrolyzed gelatin and casein, and was significantly stimulated by formate and fumarate. Growth in broth supplemented with formate and fumarate was abundant and turbidity was three times that in unsupplemented broth. The isolated strains were not stimulated to the same extent by formate and fumarate. The isolated strains must not be identified as B. corrodens. The similarity to the Campylobacter strains is greater. However, these were microaerophilic and grew in air supplemented with 30 per cent carbon dioxide. The cells of Campylobacter were spirally curved and were very actively motile with a characteristic corkscrew-like motion which was different from the more darting motion of the isolated strains. The addition of formate and fumarate stimulated the growth of Campylobacter bubulus to the same extent as the isolated strains. Campylobacter sputorum has been found in the gingival crevice flora of man (Loesche et al, 1965). No reference strain was available but form the description of the species (Loesche et al. 1965; Smibert, 1974) it is obvious that the isolated strains are similar to Campylobacter sputorum. Loesche (1968) reported that formate in an atmosphere of nitrogen or hydrogen atmosphere supported the growth of C. sputorum.

The six isolated strains in this group have recently been studied by Smibert and Holdeman(1976). They identified one of the strains (BAlla-g) as Vibrio succinogenes ("10296"). The other five strains, designated as "10278-group" were found to differ from V. succinogenes only in the shape of the cells.

There are great similarities between C. sputorum and V. succinogenes but the G+C ratio of the DNA seems to be different (Sebald and Veron, 1963; Veron and Chatelain, 1973). Recently van Palenstein Helderman and Winkler (1975) isolated strains from the human gingival crevice which were identified as C. sputorum despite the fact that they were found to have a G+C ratio of 48-50 per cent and in that respect were similar to V. succinogenes. In a later study van Palenstein Helderman (1975) designated strains of this type as "Vibrio (Campylobacter) sputorum".

It cannot be said for certain whether the strains isolated in this study are C. sputorum or V. succinogenes because no analysis of the G+C ratio of the DNA was made and no reference strains of V. succinogenes and C. sputorum were included in the taxonomic analysis. They are therefore referred to as C. sputorum which is the only one of the two species that has been reported to occur in the human oral cavity.

VEILLONELLA

This group consists of two isolated strains.

Morphology. The strains were Gram negative cocci, nonmotile, non-sporulating and anaerobic. Cells were 1 µm in diameter, spherical, appeared in clusters or irregular masses.

Colonies. On blood agar incubated anaerobically for 7 days the colonies were 2 to 3 mm in diameter, circular, entire, effuse to convex and gray.

Cultural characteristics. There was no growth in PY-glucose broth. Abundant growth occurred in PY-lactate broth and PY-pyruvate broth.

Biochemical reactions. No carbohydrates tested were fermented. Both strains reduced nitrate and produced large amounts of gas in PY-glucose agar. None of the strains hydrolyzed esculin, starch, casein, gelatin and egg protein. Ammonia, hydrogen sulfide and indol were not produced. DNAse, lipase, lecithinase were not detected. Hydrogen peroxide was decomposed by one strain.

Fermentation products. In PY-glucose broth traces of acetic and propionic acids were produced. Acetic and propionic acids were produced from pyruvate and lactate. The proportion of acetic to propionic acid was 5:1 from pyruvate and 1:1.5 from lactate.

Antibiotic sensitivity.

	Minimum inhibitory concentration (µg/ml)	
	Gl1a-e	P11b-c
Kanamycin	100	100
Neomycin	100	1 000
Penicillin G	0.01	0.01
Erythromycin	>1	>1
Tetracycline	0.1	0.1
Rifampicin	0.1	1
Polymyxin B	1	1
Bacitracin	10	10
Colistin	1	1
Metronidazol	0.1	0.1

One strain (Gl1a-e) which decomposed hydrogen peroxide is identified as Veillonella alcalenscens and the other strain (P11b-c) as Veillonella parvula.

This group consists of five isolated strains and the reference strain Peptostreptococcus anaerobius (VPI 4330-1).

Morphology. The strains were anaerobic, nonmotile, non-sporulating, Gram positive cocci, occurring in chains. Cells were often somewhat elongated. The average size was 1-1.2 µm.

Colonies. On anaerobic blood agar incubated for 5 days the colonies were 2 to 3 mm in diameter, circular, entire but sometimes with a "budding" at the margin, convex to pulvinate, opaque, white to light yellow. One strain (AB13a-c) grew with raised to low convex gray-green, translucent colonies.

Cultural characteristics. There was moderate growth in PY-broth. PY-glucose broth cultures grew abundantly. After incubation for 14 days the pH was 5.5-6.0 in PY-glucose broth. One strain (AB13a-c) was stimulated by TEM - - serum and blood. The reference strain grew on blood agar incubated in air supplemented with 30 per cent carbon dioxide.

Biochemical reactions. Glucose, fructose and maltose were fermented. None of the other tested carbonhydrates was fermented. All strains produced ammonia, hydrogen sulfide and large amounts of gas. None of the strains hydrolyzed esculin, starch, casein, gelatin, egg protein, produced acetoin, indol or reduced nitrate. Catalase, lipase and lecithinase were not detected. In two strains DNAse was detected.

Fermentation products. In PY-glucose broth all strains produced acetic acid (10-15 mM), propionic acid (1.5-5 mM), isobutyric acid (0.5-3.5 mM), butyric acid (0.5-3 mM), isovaleric acid (1-2.5 mM) and isohexanoic acid (2.5-8 mM). Three strains produced traces of valeric acid and four strains small amounts of lactic acid. The average proportions between acetic, propionic, isobutyric, butyric, isovaleric and isohexanoic acid were 8:2:2:1:1:3. The same acids, but in greater quantities were produced in PY broth. Propionic acid was produced by all strains from threonine.

Antibiotic sensitivity.

	Minimum inhibitory concentration (µg/ml)					
	VPI 4330-1	C11b-a	D13a-b	G11a-a	AB13a-c	UJB13-a
Kanamycin	10 000	10 000	10 000	10 000	1 000	1 000
Neomycin	1 000	1 000	1 000	1 000	100	1 000
Penicillin G	0.1	0.01	0.1	0.01	0.01	0.01
Erythromycin	0.01	0.01	0.01	0.01	0.01	0.1
Tetracycline	0.01	0.01	0.1	0.1	0.1	0.1
Rifampicin	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Polymyxin B	1 000	100	1 000	1 000	1 000	1 000
Bacitracin	0.1	0.01	0.1	0.1	0.1	0.01
Colistin	>1 000	1 000	>1 000	>1 000	1 000	1 000
Metronidazol	0.001	0.0001	0.0001	0.0001	0.01	0.01

The characteristics of the five strains in this group were closely related to each other and to the reference strain of Peptostreptococcus anaerobius (VPI 4330-1) and conform to the description of that species. They are therefore identified as Peptostreptococcus anaerobius.

PEPTOCOCCUS group 1

This group consists of five isolated strains.

Morphology. The strains were obligately anaerobic, nonmotile, non-sporulating small cocci (0.5 µm) and occurred singly or in short chains. They stained equivocally and both Gram negative and Gram positive cells were present. Gram negative cells were dominant.

Colonies. On anaerobic blood agar incubated for 7 days small colonies (less than 1 mm) developed. They were circular, raised or convex, translucent and gray.

Cultural characteristics. There was no visible growth in PY-glucose broth and pH was not reduced after 14 days incubation. None of the additives stimulated growth in broth.

Biochemical reactions. Carbohydrates were not fermented. Esculin, starch, casein, gelatin, egg protein were not hydrolyzed. Catalase, DNAse, lipase and lecithinase were not detected. Ammonia, hydrogen sulfide, acetoin, indol, or gas were not produced. Nitrate was not reduced.

Fermentation products. In PY-glucose broth small amounts of propionic acid (2-4 mM) were produced. The same acid and amounts were detected in PY broth cultures. Pyruvate and lactate were not utilized. Traces of propionic acid were detected in PY-threonine broth cultures.

Antibiotic sensitivity.

	Minimum inhibitory concentration (µg/ml)				
	C11b-g	P11a-i	AB13a-h	UJB13-e1	BN11a-k
Kanamycin	1	0.1	10	0.1	0.1
Neomycin	10	1	10	0.1	1
Penicillin G	0.01	0.001	0.1	0.0001	0.01
Erythromycin	0.1	1	1	0.1	1
Tetracycline	0.1	0.1	0.1	0.1	1
Rifampicin	1	1	1	1	1
Polymyxin B	0.1	0.1	10	0.1	0.1
Bacitracin	1	10	100	10	1
Colistin	0.1	0.1	100	0.1	1
Metronidazol	1	1	1	0.1	0.1

The taxonomic position of these strains is very uncertain. They are predominantly Gram negative but they do not utilize lactate or pyruvate and do not reduce nitrate and cannot be assigned to the genus Veillonella. The results to date indicate that these strains receive their energy from peptones more than from carbohydrates and they would probably fit into the genus Peptococcus or Peptostreptococcus but are not like any described species. The strains in this group were also studied at the Anaerobe Laboratory, Virginia Polytechnic Institute and they could not be identified (Holdeman personal communication). They are therefore referred to as Peptococcus group 1.

PEPTOCOCCUS group 2

This group consists of seven isolated strains.

Morphology. The strains were anaerobic, nonmotile, non-sporulating, Gram positive cocci. Cells were 0.5-1 µm and occurred in pairs or short chains.

Colonies. On anaerobic blood agar incubated for 5 days the colonies were 1 to 2 mm in diameter, circular, entire, convex, opaque and white. One strain (AC9a-c) grew with translucent, gray, low convex colonies usually less than 1 mm in diameter.

Cultural characteristics. There was moderate growth in PY-glucose broth but no reduction of the pH in any medium. None of the additives stimulated growth.

Biochemical reactions. None of the tested carbohydrates was fermented. Ammonia was produced in AMC broth by four strains. Esculin, starch, casein, gelatin, egg protein were not hydrolyzed. Catalase, DNAse, lipase and lecithinase were not detected. Indol, hydrogen sulfide or gas were not produced and nitrate was not reduced.

Fermentation products. In PY-glucose broth all strains produced acetic acid (5-20 mM) and one strain (AC9a-c) also produced lactic acid (10 mM). Almost the same amount of acetic acid was produced in PY broth as in PY-glucose broth. From pyruvate four strains produced large amounts of acetic acid (25-50 mM).

Antibiotic sensitivity.

	Minimum inhibitory concentration (µg/ml)						
	D13a-c	P11a-d	AB13a-d	AC9a-c	BA11a-e	UJB13-b	BN11a-b
Kanamycin	1	1	10	1	1	1	10
Neomycin	100	100	100	1 000	100	1 000	100
Penicillin G	0.1	0.1	0.1	1	0.01	0.1	0.1
Erythromycin	>1	1	1	>1	0.1	1	1
Tetracycline	0.1	0.1	0.1	1	0.1	1	0.01
Rifampicin	0.0001	0.001	0.0001	0.0001	0.001	0.001	0.0001
Polymyxin B	1 000	>1 000	1 000	1 000	1 000	1 000	1 000
Bacitracin	10	100	10	0.1	10	100	100
Colistin	>1 000	>1 000	>1 000	>1 000	1 000	>1 000	>1 000
Metronidazol	0.0001	0.0001	0.01	0.001	0.0001	0.0001	0.01

The characteristics of these strains conform to Peptococcaceae and because peptones or amino acids are the main energy sources they belong to the genus Peptococcus or Peptostreptococcus. The main difference between these genera is the formation of chains of cells. However, short chains may be present in both genera. The present strains formed short chains but also occurred in pairs. The strains P11a-d and UJB13-b were studied at the Anaerobe Laboratory, Virginia Polytechnic Institute and identified as Peptostreptococcus micros. (Holdeman, personal communication).The strains in this group are similar and identified as Peptostreptococcus micros.

EUBACTERIUM group 1.

This group consists of three isolated strains.

Morphology. The strains were obligately anaerobic, nonmotile, non-sporulating, Gram positive rods. Two of the strains had slender cells 0.5 by 3-15 µm and one strain (P11a-e)had shorter cells 0.75 by 1-3 µm. The rods had round ends and there was no branching.

Colonies. On anaerobic blood agar incubated for 7 days the colonies were 1.5 to 2 mm in diameter, circular, entire, umbonate, opaque, white to yellow.

Cultural characteristics. There was moderate growth in PY-glucose broth and the pH was 6.2-6.5 after 14 days incubation. None of the additives stimulated growth.

Biochemical reactions. None of the tested carbohydrates was fermented. Hydrogen sulfide was produced by all strains and ammonia by two strains. None of the strains hydrolyzed esculin, starch, casein, gelatin, egg protein, produced indol or gas. Catalase, DNAse, lipase and lecithinase were not present. Nitrate was not reduced.

Fermentation products. In PY-glucose broth acetic acid (1-2 mM), isobutyric acid (1.5 mM), isovaleric acid (1.5 mM), isohexanoic (1.5-3 mM) were produced by all strains. Two strains produced traces of lactic acid and one octanoic acid. In PY broth the same acids were produced but in greater amounts.

Antibiotic sensitivity.

	Minimum inhibitory concentration (µg/ml)		
	H9a-b	P11a-e	BN11a-a
Kanamycin	100	100	100
Neomycin	100	1 000	100
Penicillin G	0.01	0.01	0.01
Erythromycin	0.1	0.1	0.1
Tetracycline	0.01	0.001	0.01
Rifampicin	0.0001	0.0001	0.0001
Polymyxin B	1 000	1 000	1 000
Bacitracin	100	1	100
Colistin	1 000	1 000	1 000
Metronidazol	0.0001	0.01	0.1

The characteristics of these strains conform to the genus Eubacterium but not to any species in that genus and they are therefore assigned as Eubacterium group 1.

EUBACTERIUM group 2.

This group consists of three isolated strains and the reference strain Eubacterium alactolyticum (ATCC 19301).

Morphology. The strains were anaerobic nonmotile, non-sporulating, Gram positive rods. The cells were 1 by 3-5 µm, slightly curved, with rounded ends, often in pairs and non-branching.

Colonies. On anaerobic blood agar incubated 5 days the colonies were 1 to 2 mm in diameter, circular, entire, convex to pulvinate, smooth, shiny. Smaller colonies were translucent, larger opaque and white-yellow.

Cultural characteristics. There was good growth with granular sediment in PY-glucose broth. Terminal pH was 5.5-5.8. In PY-fructose broth terminal pH was 4.8-5.6.

Biochemical reactions. Fructose, glucose and mannitol were fermented. None of the other tested carbohydrates was fermented. Large amounts of gas were produced. Esculin, starch, casein, gelatin, egg protein were not hydrolyzed. Catalase, DNAse, lipase and lecithinase were not detected. Ammonia, acetoin, hydrogen sulfide, indol were not produced. Nitrate was not reduced.

Fermentation products. In PY-glucose broth acetic acid (1.5-3 mM), butyric acid (1-3 mM) and hexanoic acid (10-17 mM) were produced. Traces of succinic acid was produced by one strain and octanoic by another strain. The average proportions between acetic, butyric and hexanoic acids was 1:1:6. In PY-lactate broth butyric acid and hexanoic acid were produced and in PY-pyruvate broth acetic acid, butyric acid and hexanoic acid .

Antibiotic sensitivity.

	Minimum inhibitory concentration (µg/ml)			
	ATCC19301	C11b-d	AB13a-n	AC-a
Kanamycin	10 000	10 000	10 000	10 000
Neomycin	1 000	1 000	1 000	1 000
Penicillin G	0.01	0.01	0.01	0.001
Erythromycin	0.1	0.1	0.1	0.1
Tetracycline	1	0.1	1	1
Rifampicin	0.001	0.0001	0.0001	0.0001
Polymyxin B	1 000	1 000	1 000	1 000
Bacitracin	0.1	0.1	1	0.1
Colistin	1 000	1 000	1 000	1 000
Metronidazol	0.001	0.0001	0.1	0.1

Cell wall components. The cell wall of the isolated strains contained DL-diaminopimelic acid, galactose, glucose, rhamnose (Bowden, personal communication).

The characteristics of these strains indicates that they belong to the genus Eubacterium. Holdeman and Moore (1972, 1974) report the presence of DL-diaminopimelic acid in the cell wall of E.alactolyticum. Edwardsson and Bowden (1974) report that the reference strain E.alactolyticum ATCC 19301 has DL-diaminopimelic acid, galactose, glucose and rhamnose in its cell wall. The isolated strains clustered with that reference strain and have the same cell wall composition and are identified as E.alactolyticum.

These groups consist of seven isolated strains and the reference strain Eubacterium lentum (ATCC 25559).

Morphology. The strains were anaerobic, nonmotile, non-sporulating, Gram positive rods. Cells were 0.5 by 1-1.5 µm, straight, with rounded ends, single or in short chains, non-branching (Fig. IV:1).

Colonies. On anaerobic blood agar incubated 7 days the colonies were 0.75 to 1.5 mm, circular, entire, raised to low convex, translucent to semi-opaque, gray-white.

Cultural characteristics. There was slight growth without visible turbidity in PY-glucose and pH was not changed. Growth was not stimulated by any additive.

Biochemical reactions. None of the tested carbohydrates was fermented. The four strains in group 3 produced ammonia in L-arginine broth and three of the strains in that group reduced nitrate. None of the strains in group 4 produced ammonia or reduced nitrate. None of the strains in the two groups hydrolyzed esculin, starch, casein, gelatin, egg protein, produced acetoin, hydrogen sulfide, indol or gas. Catalase, DNAse, lipase and lecithinase were not detected in any strain.

Fermentation products. In Py- glucose broth two strains produced small amounts of acetic acid (1-1.5 mM) an one strain traces of lactic acid. None of the other strains produced any detectable fermentation product in any of the other analyzed media.

Antibiotic sensitivity.

	Minimum inhibitory concentration (µg/ml)							
	ATCC 25559	C11b-c	AC-b	BN11a-e	P9a-h	AB13a-1	BA11a-f	UJB13-d
Kanamycin	1	1	1	0.1	100	100	100	100
Neomycin	1	1	1	1	100	1 000	100	100
Penicillin G	1	0.1	0.1	0.1	0.01	0.01	0.1	0.01
Erythromycin	0.1	0.001	0.001	0.001	0.1	0.1	0.1	0.1
Tetracycline	1	1	1	1	0.1	1	0.1	0.1
Rifampicin	0.001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Polymyxin B	1 000	100	1 000	100	100	1 000	1 000	100
Bacitracin	10	0.01	0.01	0.01	10	10	10	10
Colistin	1 000	1 000	1 000	100	1 000	1 000	1 000	1 000
Metronidazol	0.1	0.01	0.001	0.1	0.0001	0.1	0.001	0.001

The results indicate that the strains belong to the genus Eubacterium. Strains in group 3 clustered with the reference strain E.lentum (ATCC 25559) and are similar to that strain. Recently Sperry and Wilkins (1976) reported that E. lentum produces ammonia from L-arginine and that growth is enhanced in that medium in contrast to other "biochemically inactive" Gram positive rods. The strains in group 3 have this characteristic and are identified as E.lentum. The four strains in group 4 can only be identified as Eubacterium species and are designated Eubacterium group 4.

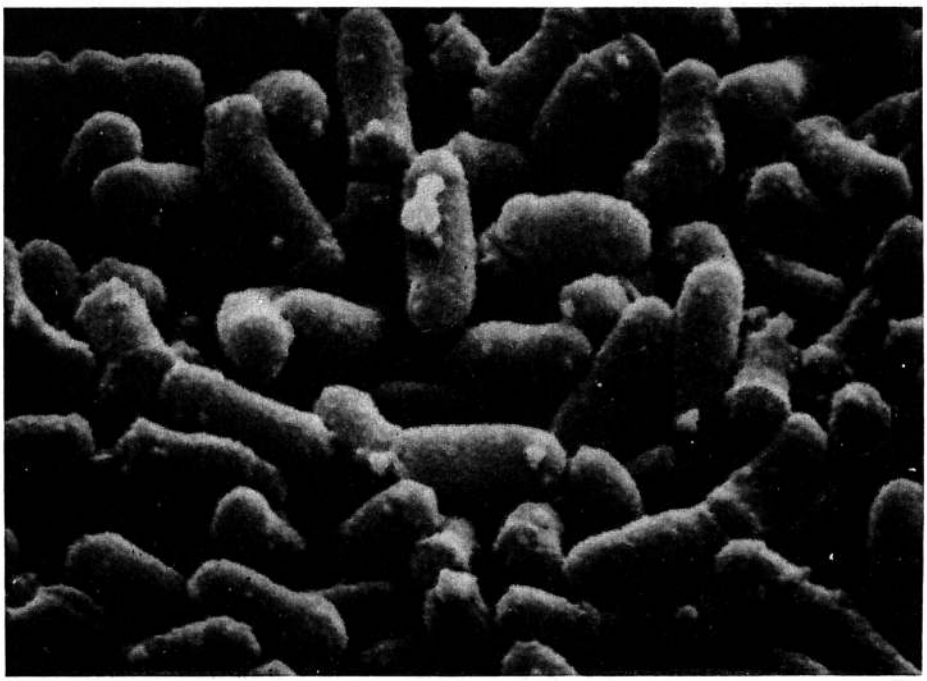

Fig IV:1. Scanning electron micrograph of a colony of the strain AB13a-1. Original magnification, X 24 000.

This group consists of one isolated strain and the reference strain Propionibacterium acne (NCTC 737).

Morphology. The strains were nonmotile, non-sporulating, Gram positive, pleomorphic rod. Cells were extremely variable in size and form, but without branching. The dominant cells were 1 by 3-5 µm.

Colonies. On anaerobic blood agar incubated for 5 days the colonies were 2 mm, circular, entire, pulvinate, opaque and white.

Cultural characteristics. There was abundant growth in PY-glucose broth and the terminal pH was 4.8-5.2. Tween 80 and blood stimulated the growth of the isolated strain. Both strains grew on blood agar incubated in air supplemented with 30 per cent carbon dioxide but not in air alone.

Biochemical reactions. The characteristics of the strains are given in Table IV:6. Arabinose, cellobiose, dulcitol, erythritol, esculin, inositol, inulin, melezitose, ribose, salicin, sorbose and starch were not fermented. DNAse, lipase and lecithinase were not detected.

Fermentation products. In PY-glucose broth acetic acid and propionic acid were produced in the average proportions 1:3. The same acids were produced in PY-pyruvate broth and PY-lactate broth. The average proportions were in pyruvate 1:1 and in lactate 1:2.

Antibiotic sensitivity.

	Minimum inhibitory concentration (µg/ml)	
	NCTC 737	X11a-b
Kanamycin	100	100
Neomycin	10	10
Penicillin G	0.01	0.01
Erythromycin	0.01	0.01
Tetracycline	1	0.1
Rifampicin	0.01	0.0001
Polymyxin B	100	100
Bacitracin	0.1	1
Colistin	1 000	100
Metronidazol	>10	>10

Cell wall components. The cell wall contained LL-diaminopimelic acid, galactose and glucose (Bowden, personal communication).

The strain is identified as Propionebacterium acne and the presence of galactose in the cell wall suggests that it belongs to serotype 1 (Moore and Holdeman, 1974).

Table IV:6

Characteristics of strains assigned to Propionibacterium and Arachnia groups.

Characteristics	PROPIONIBACTERIUM		ARACHNIA			
	P. acne		A. propionica			
	NCTC 737	X11a-b	ATCC 14157	A 41	P9a-f	U13a-a
Products from PYG broth[1]	A,P	A,P	A,P,s	A,P,s	a,P,s	A,P,s
Products from lactate	a,p	A,P	A,P	A,P	-	A,P
Threonine → propionate	+	+	+	+	+	+
Acid produced from:[2]						
Fructose	a	a	a	a	a	a
Glucose	a	a	a	a	a	a
Glycerol	-	-	-	-	w	-
Lactose	-	-	-	w	a	a
Maltose	-	-	a	a	a	a
Mannitol	-	-	a	a	a	a
Mannose	a	w	a	-	a	a
Raffinose	-	-	a	a	a	a
Rhamnose	-	-	-	w	-	-
Sorbitol	-	-	-	a	a	a
Sucrose	-	-	w	a	a	a
Trehalose	-	-	-	a	a	a
Xylose	-	-	-	-	w	-
Catalase present	+	+	-	-	-	-
Gelatin hydrolyzed	+	+	-	-	-	-
Esculin hydrolyzed	-	-	-	-	-	+
Starch hydrolyzed	-	-	-	-	-	-
Casein hydrolyzed	+	+	+	-	+	+
Indol produced	+	+	-	-	-	-
Nitrate reduced	+	+	+	+	+	+
Ammonia produced in AMC broth	-	+	-	-	-	-
Hydrogen sulfide produced	-	-	-	-	-	-
Acetoin produced	-	-	+	+	+	+
Gas produced	-	-	-	-	-	-

1) Products from PYG broth: capital letters indicate ≥10 mM; small letters <10 mM; A, acetic acid; P, propionic acid; S, succinic acid.

2) a, pH 5.5 or below; w, pH 5.6-5.8; -, pH above 5.8. +, positive reaction; -, negative reaction.

This group consists of two isolated strains and the reference strains Arachnia propionica (ATCC 14157, A 41).

Morphology. The strains were nonmotile, non-sporulating, Gram positive rods. Cells were pleomorphic but dominant cells were 1 by 2-5 µm. Branching occurred. Branching filaments of 50-100 µm length were present and also swollen coccoid cells.

Colonies. On anaerobic blood agar incubated for 7 days the colonies were 2 to 3 mm, circular, entire, pulvinate, opaque, white to yellow and smooth.

Cultural characteristics. In PY-glucose broth there was abundant growth and the terminal pH was 5.0-5.4. At primary isolation none of the isolated strains grew in air. After serial transfers and with a heavy inoculum one of the isolated strains grew weakly in air. The reference strains grew in air. All strains grew in air supplemented with 30 per cent carbon dioxide.

Biochemical reactions. The characteristics of the strains are given in Table IV:6. None of the strains fermented arabinose, cellobiose, dulcitol, erythritol, esculin, inositol, inulin, melezitose, melibiose, ribose, salicin, sorbose, starch. DNAse, lipase and lecithinase were not detected.

Fermentation products. In PY-glucose broth acetic acid (8-16 mM), propionic acid (16-32 mM) and trace amounts of succinic acid were produced. The average proportions between acetic and propionic acid were 1:2. The reference strains and one of the isolated strains utilized pyruvate and lactate and produced acetic and propionic acids.

Antibiotic sensitivity.

	Minimum inhibitory concentration (µg/ml)			
	ATCC 14157	A 41	P9a-f	U13a-a
Kanamycin	1 000	100	100	100
Neomycin	100	1 000	100	1 000
Penicillin G	0.1	0.1	0.01	0.01
Erythromycin	0.1	1	0.1	0.1
Tetracycline	0.1	1	0.1	0.1
Rifampicin	0.001	0.0001	0.0001	0.0001
Polymyxin B	1 000	1 000	1 000	1 000
Bacitracin	1	1	10	1
Colistin	1 000	1 000	1 000	1 000
Metronidazol	>10	>10	>10	>10

Cell wall components. The cell walls contained LL-diaminopimelic acid, galactose and glucose (Bowden, personal communication).

On the basis of the fermentation products the isolated strains belong to either of the genera Arachnia or Propionibacterium. The genus Arachnia differs from the genus Propionibacterium primarily in the consistent absence of catalase, the formation of filamentous cells (Pine and Georg, 1974) and in fermentation of raffinose, lactose (Moore and Holdeman, 1974; Pine and Georg, 1974). In contrast to Propionibacterium, Arachnia do not ferment pyruvate or lactate.

In the nucerical taxonomic analysis the two isolated strains in this group (P9a-f, U13a-a) were similar at a 93 per cent level. The average similarity to the two reference strains of the species Arachnia propionica was 86 per cent and to the reference strain of the species Propionibacterium acne 76 per cent.

The two isolated strains in this group were catalase negative, had filamentous cells and fermented lactose and raffinose. In polythetic comparison the similarity was greater to the relevant species of the genus Arachnia than to the species in the genus Propionibacterium. Pyruvate and lactate were fermented by one of the strains. In the present study both reference strains did ferment pyruvate and lactate and these characteristics are obviously not good criteria for differentiating between the genera. The isolated strains are identified as Arachnia propionica.

LACTOBACILLUS group 1

This group consists of five isolated strains.

Morphology. The strains were Gram positive rods, nonmotile, non-sporulating and obligately anaerobic. Cells were pleomorphic but all cells were rods 0.5-1 by 2-6 µm. Swollen cells were common, bifid cells occurred sparsely.
Colonies. On blood agar incubated anaerobically for 7 days the colonies were 0.75 to 1.5 mm, effuse or raised, circular, entire, translucent, gray-white.
Cultural characteristics. There was very poor growth in PY-glucose broth. When this medium was supplemented with Tween 80 abundant growth occurred. The terminal pH was 5.0-5.4. The addition of TEM and serum stimulated the growth of three strains.
Biochemical reactions. The characteristics are given in Table IV:7. Dulcitol, erythritol, glycerol, inositol, inulin, mannitol, melezitose, melibiose, raffinose, rhamnose, ribose, sorbitol, sorbose were not fermented. Catalase, DNAse, lipase and lecithinase were not present. Gelatin was not hydrolyzed. Hydrogen sulfide, gas,acetoin, and indol were not produced. Ammonia was produced in L-arginine broth but not in AMC broth. Nitrate was not reduced.
Fermentation products. In PY-glucose broth acetic acid (2.5-10 mM) and lactic acid (16-45 mM) were produced by all strains. Four strains also produced ethanol (2-20 mM). The proportions of acetic and lactic acid varied from 1:2 to 1:10. No succinic acid or propionic acid was detected in any medium.
Antibiotic sensitivity.

	Minimum inhibitory concentration (µg/ml)				
	D13a-d	G11a-f	AB13a-m	AC-d	BA11a-c
Kanamycin	10 000	10 000	10 000	10 000	10 000
Neomycin	1 000	1 000	1 000	100	1 000
Penicillin G	0.1	0.001	0.001	0.001	0.01
Erythromycin	0.1	0.1	0.1	0.01	0.1
Tetracycline	0.1	1	1	1	10
Rifampicin	10	10	10	10	10
Polymyxin B	1 000	1 000	1 000	1 000	1 000
Bacitracin	10	0.1	10	0.1	0.1
Colistin	1 000	1 000	1 000	1 000	1 000
Metronidazol	0.1	0.1	1	0.001	0.1

Cell wall composition. All strains had ornithine and lysine in their cell walls. The carbohydrates in the cell wall were AC-d, galactose, glucose; BA11a-c, galactose, glucose, rhamnose; D13a-d, galactose; AB13a-m, glucose, rhamnose; G11a-f, glucose (Bowden, personal communication).

This is a rather heterogeneous group which contains strains stimulated by Tween 80 and producing lactic acid as the main end product when glucose is fermented. Ammonia is produced from L-arginine by all strains. The results indicate that the strain belongs to either of the genera Lactobacillus or Bifidobacterium. The proportions of the fermentation products indicate that the strains belong to the genus Lactobacillus. The characteristics do not conform to any described species in that genus and the strains are tentatively identified as Lactobacillus species and designed Lactobacillus group 1. When characterized at Virginia Polytechnic Institute, USA the strain AB13a-m was found to be similar to nine strains which had been isolated from abdominal abcesses, lung, actinomycosis, purulent pleuresy, rectal abscess and periodontal abscess (Holdeman, personal communication).

Table IV:7 Characteristics of strains assigned to Lactobacillus groups.

Characteristics	LACTOBACILLUS group 1					LACTOBACILLUS group 2		LACTOBACILLUS group 3	
						L. catenaforme			
	AC-d	BA11a-c	D13a-d	AB13a-m	G11a-f	ATCC 25536	AB13a-b	UJB13-f[3] BN11a-h	C11b-f H11a-c AB13a-e
Products from PYG broth[1]	e,a,L	e,a,L	e,A,L	a,L	e,a,L	e,a,L	e,a,L	e,a,L	e,a,L
Acid produced from[2]									
Arabinose	-	-	-	-	-	-	-	-	w(1)
Cellobiose	-	-	-	-	a	a	a	a	-
Esculin	-	-	-	-	w	a	-	w(1)	-
Fructose	a	a	-	a	a	a	a	a	a
Glucose	a	a	a	a	a	a	a	a	a
Lactose	-	-	-	-	-	a	a	-	-
Maltose	w	-	a	a	a	w	-	a	a
Mannose	-	-	-	-	-	a	a	a	a
Salicin	-	-	-	-	a	a	a	-	a(1)
Starch	-	-	-	a	a	a	a	-	-
Sucrose	a	a	-	a	a	a	a	a	a
Trehalose	-	-	-	-	-	w	-	a	a(1)
Xylose	-	-	-	-	-	-	-	w	w(1)
Esculin hydrolyzed	-	-	-	-	+	+	+	+	+
Casein hydrolyzed	-	-	-	-	-	+	+	-	-
Starch hydrolyzed	-	-	-	+	-	+	+	-	-
Ammonia produced in L-arginine broth	+	+	+	+	+	-	-	-	+

1) Products from PYG broth: capital letters indicate ≥10 mM; small letters <10 mM; A, acetic acid; L, lactic acid; E, ethanol.

2) a, pH in broth 5.5 or below; w, pH 5.6-5.8; - pH above 5.8.

3) Numbers in parentheses denote number of strains fulfilling the listed critiera; +, positive reaction; -, negative reaction.

LACTOBACILLUS group 2

This group consists of one isolated strain and the reference strain Lactobacillus catenaforme (ATCC 25536).

Morphology. The strains were anaerobic, nonmotile, non-sporulating, Gram positive rods. The cells were 1 by 3-8 µm, with rounded ends, non-branching, and occurred often in short chains.

Colonies. On blood agar incubated for 7 days the colonies were 2 to 3 mm, circular, umbonate or pulvinate, opaque and white.

Cultural characteristics. Abundant growth occurred in PY-glucose broth. The terminal pH was 4.5-4.8. None of the additives enhanced growth.

Biochemical reactions. The characteristics are given in Table IV:7. Dulcitol, erythritol, glycerol, inositol, inulin, mannitol, melezitose, melibiose, raffinose, rhamnose, ribose, sorbitol, sorbose were not fermented. Catalase, DNAse, lipase and lecithinase were not present. Gelatin was not hydrolyzed. Ammonia, hydrogen sulfide, gas,acetoin were not produced. Nitrate was not reduced.

Fermentation products. In PY-glucose broth acetic acid (5 mM) and lactic acid (25 mM) and ethanol (3 mM) were produced. Pyruvate and lactate were not utilized.

Antibiotic sensitivity.

	Minimum inhibitory concentration (µg/ml)	
	ATCC 25536	AB13a-b
Kanamycin	1 000	10 000
Neomycin	>1 000	>1 000
Penicillin G	0.001	0.01
Erythromycin	0.1	1
Tetracycline	0.01	1
Rifampicin	10	10
Polymyxin B	1 000	1 000
Bacitracin	1	1
Colistin	1 000	1 000
Metronidazol	1	10

Cell wall composition. The cell wall of the isolated strain contained lysine and glucose (Bowden, personal communication).

The isolated strain is similar to the reference strain L. catenaforme, (ATCC 25536),(Moore and Holdeman, 1973) and is identified as belonging to that species.

LACTOBACILLUS group 3

This group consists of five isolated strains.

Morphology. The strains were Gram positive, nonmotile, non-sporulating and obligately anaerobic. Cells varied and both coccoid forms and rods 0.75 by 2-5 µm were present. Cells occurred singly or in chains and often had swellings at the midpoint giving the cells in older broth cultures a bubble-shaped appearance.

Colonies. On blood agar incubated for 7 days the colonies were 1 to 1.5 mm, raised with concave bevelled edges, circular, entire, translucent, gray.

Cultural characteristics. Very slight growth occurred in PY-glucose broth. When this medium was supplemented with Tween 80 abundant growth occurred. The terminal pH was 4.0-4.8. Addition of TEM-serum also stimulated the growth of all strains and blood and serum stimulated two strains (C11b-f, H11a-c).

Biochemical reactions. The characteristics are given in Table IV:7. Dulcitol, erythritol, glycerol, inositol, inulin, mannitol, melezitose, melibiose, raffinose, rhamnose, ribose, sorbitol, sorbose were not fermented. Catalase, DNAse, lipase and lecithinase were not present. Gelatin was not hydrolyzed. Hydrogen sulfide, gas, acetoin and indol were not produced. Nitrate was not reduced.

Fermentation products. From PY-glucose broth all strains produced ethanol (3.5-6 mM), acetic acid (1-4 mM) and lactic acid (25-60 mM). The average proportions between acetic and lactic acid were 1:15.

Antibiotic sensitivity.

	Minimum inhibitory concentration (µg/ml)				
	C11b-f	H11a-c	AB13a-e	UJB13-f	BN11a-h
Kanamycin	10	10	10	1	1
Neomycin	100	10	100	100	100
Penicillin G	0.1	0.1	0.1	0.1	0.1
Erythromycin	0.001	0.001	0.001	0.001	0.0001
Tetracycline	0.1	0.1	1	1	1
Rifampicin	0.0001	0.0001	0.0001	0.0001	0.0001
Polymyxin B	1 000	1 000	1 000	1 000	1 000
Bacitracin	10	10	1	10	1
Colistin	1 000	1 000	1 000	1 000	1 000
Metronidazol	10	1	1	10	1

Cell wall composition. Two strains (UJB13-f, BN11a-h) had lysine, galactose and glucose in the cell walls. Three strains (C11b-f, H11a-c, AB13a-e) had ornithine, lysine, galactose, glucose and rhamnose in the cell wall (Bowden, personal communication).

The strains in this group differ from the strain in Lactobacillus group 1 in the proportion of the fermentation products, sensitivity to rifampicin, production of acid from mannose, and hydrolysis of esculin. Within the group UJB13-f and BN11a-h differ from the other strains in fermenting cellobiose and do not produce ammonia from arginine. In addition they have no ornithine and rhamnose in the cell walls. The cells of these two strains were predominately rod shaped. The strains C11b-f, H11a-c, AB13a-e had coccoid cells in long chains but also rod shaped cells (Fig IV:2).

The production of lactic acid as the major fermentation product assigns these strains to the genus Lactobacillus. However, the characteristics do not conform to any described species of the genus. The strains are designated Lactobacillus group 3.

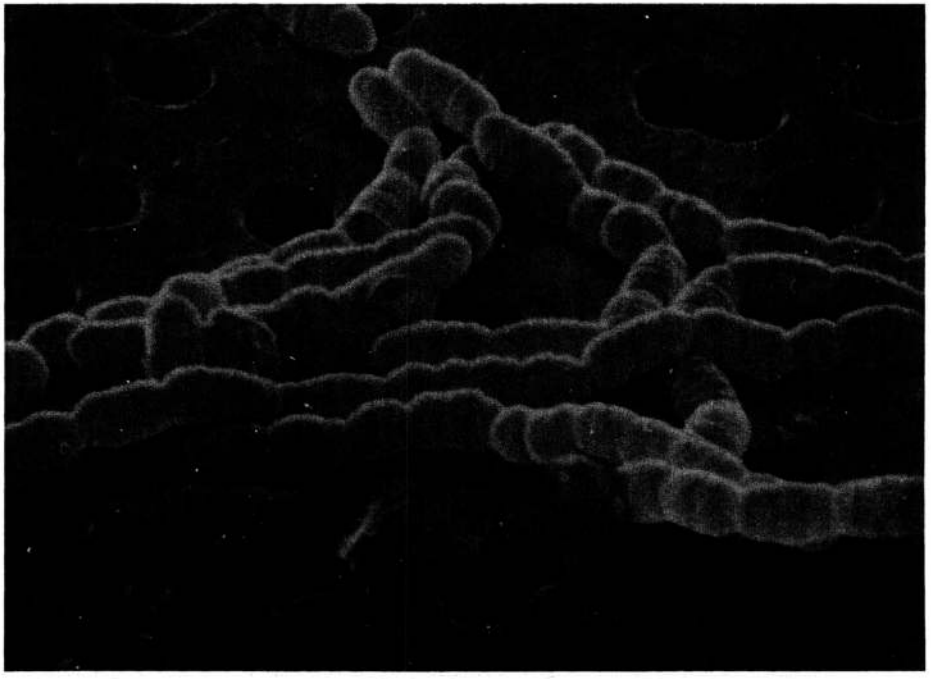

Fig IV:2. Scanning electron micrograph of a broth culture (24 h.) of the strain H11a-c. Original magnification, X 12 000.

The group consists of two isolated strains and the reference strains Actinomyces naeslundii (ATCC 12104) and Actinomyces viscosus (ATCC 15987).

Morphology. The strains were Gram positive rods, nonmotile, non-sporulating. Cells were pleomorphic but all cells were rods 1 by 4-10 µm. Branched cells but no filaments occurred.

Colonies. On blood agar incubated anaerobically for 5 days the colonies were 2 to 3 mm, convex to pulvinate, circular, entire, opaque white to light yellow. One of the isolated strains (X9a-a) formed red colonies when grown in air supplemented with 30 per cent carbon dioxide.

Cultural characteristics. Abundant growth occurred in PY-glucose broth. The terminal pH was 4.5-5.0. None of the additives enhanced growth. All strains grew in air supplemented with 30 per cent carbon dioxide. After several subcultures one of the isolated strains (X9a-a) grew weakly in air when a heavy inoculum was used. The reference strains grew in air.

Biochemical reactions. The characteristics are given in Table IV:8. Dulcitol, erythritol, sorbose were not fermented. Lipase and lecithinase were not present; starch was not hydrolyzed; indol, gas, hydrogen sulfide and ammonia in AMC broth were not produced.

Fermentation products. In PY glucose broth acetic acid (3.5-7 mM), lactic acid (7-25 mM) and succinic acid (5-20 mM) were produced. The reference strains produced acetic acid (2 mM) and succinic acid (10-20 mM) and A. naeslundii also lactic acid.

Antibiotic sensitivity.

	Minimum inhibitory concentration (µg/ml)			
	ATCC 12104	ATCC 15987	BA11a-h	X9a-a
Kanamycin	10	10	10	10
Neomycin	1 000	100	10	10
Penicillin G	0.01	0.1	0.01	0.01
Erythromycin	0.1	0.1	0.1	0.001
Tetracycline	0.1	1	1	0.1
Rifampicin	0.0001	0.0001	0.0001	0.0001
Polymyxin B	1 000	1 000	1 000	1 000
Bacitracin	0.1	1	0.01	1
Colistin	1 000	1 000	1 000	1 000
Metronidazol	>10	10	>10	>10

Cell wall composition. The isolated strains had ornithine, lysine and rhamnose in their cell walls. BAlla-h also had galactose and traces of fructose and X9a-a glucose, 6-deoxytalose and traces of mannose (Bowden, personal communication).

The two isolated strains in this group are allocated to the genus Actinomyces because of their fermentation products. They cluster with the reference strain of the species A. naeslundii (ATCC 12104) and A. viscosus (ATCC 15987). These two species are similar and A. viscosus is thought to be a catalase positive variant of A. naeslundii (Gerencser and Slack, 1976; Holmberg and Hallander, 1973). The definitions of these two species indicate that a wide range of organisms may be included in the species. The two isolated strains were catalase negative and are therefore identified as A. naeslundii.

Table IV:8

Characteristics of strains assigned to Actinomyces groups.

Characteristics	ACTINOMYCES group 1				ACTINOMYCES group 2				
	A. naeslundii			A. viscosus	A. odontolyticus	A. israelii			
	ATCC 12104	BA11a-h	X9a-a	ATCC 15987	NCTC 9935	ATCC 12102	G9a-c1	D13a-a	P11b-b
Products from PYG broth[1]	a,L,S	a,L,S	a,l,s	a,S	a,S	a,S	a,S	a,S	a,S
Acid produced from[2]									
Arabinose	w	-	-	-	-	-	-	a	a
Cellobiose	a	a	w	w	-	-	-	-	-
Esculin	a	a	-	-	-	-	-	-	-
Fructose	a	a	a	a	w	a	a	a	a
Glucose	a	a	a	a	a	a	a	a	a
Glycerol	-	-	-	w	-	-	-	w	w
Inositol	a	a	a	w	-	-	-	-	-
Inulin	a	-	a	a	-	-	-	-	-
Lactose	a	a	a	a	w	a	a	a	a
Maltose	a	a	a	a	-	-	w	a	a
Mannitol	-	a	-	-	-	-	-	-	-
Mannose	a	a	a	a	-	-	-	-	-
Melezitose	w	a	-	w	-	-	-	-	-
Melibiose	a	a	a	a	-	-	-	-	-
Raffinose	a	a	a	a	-	-	-	-	w
Rhamnose	w	w	-	w	-	-	-	-	-
Ribose	a	a	-	w	-	-	-	a	a
Salicin	a	a	a	w	-	-	-	-	-
Sorbitol	-	-	-	w	-	-	-	-	-
Starch	w	a	-	-	-	-	-	a	w
Sucrose	a	a	a	a	-	a	a	a	a
Trehalose	a	a	a	-	-	-	-	-	-
Xylose	a	a	-	w	-	-	a	a	a
Catalase present	-	-	-	+	-	-	+	-	-
Esculin hydrolyzed	+	+	+	+	-	-	+	-	-
Casein hydrolyzed	+	-	+	+	+	+	+	+	+
Nitrate reduced	+	+	+	+	+	+	+	-	-
Ammonia produced in L-arginine broth	+	+	+	-	-	-	-	-	-
Acetoin produced	-	+	-	-	+	+	+	+	+
DNAse produced	-	-	-	-	-	-	+	+	+

1) Products from PYG broth: capital letters indicate ≥10 mM; small letters <10 mM; A, acetic acid; L, lactic acid; S, succinic acid.

2) a, pH 5.5 or below; w, pH 5.6-5.8; -, pH above 5.8
+, positive reaction; -, negative reaction

ACTINOMYCES group 2

The group consists of three isolated strains and the reference strains Actinomyces odontolyticus (NCTC 9935) and Actinomyces israelii (ATCC 12102).

Morphology. The strains were Gram positive rods, nonmotile, non-sporulating. The cells were pleomorphic rods 1 by 2-10 μ, often arranged in V and Y forms. Branching occurred in one of the isolated strains (G9a-c1) and in the reference strains.

Colonies. On anaerobic blood agar incubated for 5 days the colonies were 2 to 3 mm, circular, entire, convex to umbonate, opaque, white and smooth. One of the isolated strains (G9a-c1) and the reference strains grew with red colonies in air supplemented with 30 per cent carbon dioxide.

Cultural characteristics. Abundant growth occurred in PY-glucose broth. The terminal pH was 4.8-5.2. None of the additives enhanced growth. All strains grew in air supplemented with 30 per cent carbon dioxide. The reference strains also grew in air.

Biochemical reactions. The characteristics are given in Table IV:8. Dulcitol, erythritol, sorbose were not fermented. Lipase and lecithinase were not present; starch was not hydrolyzed; indol, gas, hydrogen sulfide and ammonia in AMC broth were not produced.

Fermentation products. In PY-glucose broth acetic acid (2-9 mM) and succinic acid (5-25 mM) were produced. The proportions for the isolated strains were 2:1, 1:2, 1:6 and for the reference strains 1:10. No lactic acid was formed by any of the strains. From PY-pyruvate broth the strain G9a-c1 produced large amounts of acetic acid (32-40 mM) the other strains only traces.

Antibiotic sensitivity

	Minimum inhibitory concentration (μg/ml)				
	NCTC 9935	ATCC 12102	D13a-a	G9a-c1	P11b-b
Kanamycin	100	100	100	10	100
Neomycin	100	1 000	1 000	100	>1 000
Penicillin G	0.01	0.01	0.01	0.01	0.001
Erythromycin	0.01	0.001	0.001	0.001	0.001
Tetracycline	0.1	1	0.1	0.1	0.1
Rifampicin	0.0001	0.0001	0.0001	0.0001	0.0001
Polymyxin B	1 000	1 000	1 000	100	1 000
Bacitracin	1	1	1	0.1	1
Colistin	1 000	1 000	1 000	1 000	1 000
Metronidazol	10	>10	10	10	10

Cell wall composition. All isolated strains had ornithine,lysine and rhamnose in their cell walls. G9a-cl also had glucose and 6-deoxytalose. Fructose and mannose were present in the cell walls of the other two strains (Bowden, personal communication).

The production of succinic acid and acetic acid indicates that the three isolated strains belong to the genus Actinomyces. They cluster with the reference strains of the species A. odontolyticus (NCTC 9935) and A. israelii (ATCC 12102). Cell wall analysis of A. israelii has shown that the species has only galactose as sugar in the cell wall (Pine and Georg, 1965; Boone and Pine, 1968; Edwardsson and Bowden, 1974). None of the isolated strains has the cell wall pattern of A. israelii. One strain (G9a-cl) bears a great similarity to the reference strain A. odontolyticus but has catalase and cannot be assigned to that species. The strains are therefore described as Actinomyces group 2.

The assignment of the 5 strains in group 1 and group 2 into the genus Actinomyces may be based on the analysis of the fermentation products. The allocation of the strains to the genus was strenghtened when, in the numerical taxonomic analysis, they clustered with the reference strains of the genus. The further assignment into species of the genus is based on some commonly accepted characteristics. For strains not typical of accepted species cell wall analysis and serological studies may facilitate the identification. However, many isolated Actinomyces strains cannot be placed in any of the recognized species even using serological methods and a modification of the classification of actinomyces is proposed (Gerencser and Slack, 1976). It has also recently been reported that it was not possible to identify 62 oral Gram positive rods, even when 524 characteristics were used in a numerical taxonomic analysis (Spellman et al., 1974). The designation of the strains into Actinomyces species in this study is therefore tentative.

V. GENERAL DISCUSSION

Eighty-eight bacterial strains were isolated from 18 pulp chambers of teeth with periapical destruction (Table V:1). The following reasons support the assumption that these bacteria did emanate from the pulp chambers: no bacteria were isolated from teeth without radiographical signs of periapical destruction; bacteria were not found in samples obtained during preparation of the entrance to the pulp chambers; no facultatively anaerobic lactobacilli, Streptococcus salivarius or other readily cultivated bacteria of the indigenous oral flora were found.

More than 90 per cent of the isolated strains were anaerobic. This is a higher proportion of anaerobic bacteria than has previously been isolated from necrotic pulps (Brown and Rudolph, 1957; Macdonald et al., 1957; Möller, 1966; Bergenholtz, 1974). One reason for this high proportion could be that the present anaerobic technique was relatively successful, but it could also be that strict criteria were used in the selection of the teeth. A possible explanation for the higher proportion of anaerobic bacteria in the present study than in those of Kantz and Henry (1974) and Wittgow and Sabiston (1975), who also used an advanced anaerobic technique, might be that teeth with caries or restorations might have been included in these studies. Bacteria are present in the dentinal tubules of the advancing front of the carious lesion and have been shown to exist in the dentinal tubules under restorations (Brännström and Nyborg, 1971). One possible entrance route for bacteria into the pulp chambers of these teeth could be via the dentinal tubules, whereas the route into teeth with intact crowns is unknown. Identical finding in the isolated flora between the studies is than not necessarily to be expected. The possibility of picking up bacteria during the preparation of an entrance to the

Table V:1

Bacterial flora of the infected teeth.

Tooth (code)	Approximate no. of bact. per sample	Strain identification	per cent of the flora
B[1]	$<10^2$	Bacteroides ochraceus	-
C	10^3	Peptostreptococcus anaerobius	15
		Eubacterium lentum	15
		Eubacterium alactolyticum	15
		Fusobacterium sp. group 2	25
		Lactobacillus sp. group 3	15
		Peptococcus sp. group 1	15
D	10^5	Actinomyces sp. group 2	10
		Peptostreptococcus anaerobius	25
		Peptostreptococcus micros	25
		Lactobacillus sp. group 1	25
		B. melaninogenicus ss. intermedius	10
		Campylobacter sputorum	5
E	10^3	Streptococcus mitis	100
G	10^4	Peptostreptococcus anaerobius	5
		Streptococcus mitis	2
		Actinomyces sp. group 2	1
		Bacteroides sp. group 3	
		B. melaninogenicus ss. intermedius	2
		Veillonella alcalescens	80
		Lactobacillus sp. group 1	10
H	10^4	Fusobacterium sp. group 1	20
		Eubacterium sp. group 1	15
		Lactobacillus sp. group 3	55
		Fusobacterium sp. group 2	5
		B. melaninogenicus ss. asaccharolyticus	3
		Campylobacter sputorum	2
M	10^3	Streptococcus mitis	100
P	10^5	Fusobacterium sp. group 1	3
		Actinomyces sp. group 2	1
		Veillonella parvula	5
		Peptostreptococcus micros	5
		Eubacterium sp. group 1	2
		Arachnia propionica	1
		Campylobacter sputorum	70
		Eubacterium sp. group 4	2
		Peptococcus sp. group 1	10
		B. melaninogenicus ss. intermedius	1
U	10^3	Arachnia propionica	100
X	10^3	Actinomyces naeslundii	95
		Propionibacterium acne	5
AB	10^5	Fusobacterium sp. group 1	5
		Lactobacillus catenaforme	5
		Peptostreptococcus anaerobius	10
		Peptostreptococcus micros	10
		Lactobacillus sp. group 3	10
		B. melaninogenicus ss. intermedius	25
		Fusobacterium sp. group 1	5
		Peptococcus sp. group 1	10
		Campylobacter sputorum	<1
		Eubacterium sp. group 4	15
		Lactobacillus sp. group 1	5
		Eubacterium alactolyticum	<1
AC	10^2	Eubacterium alactolyticum	-[2]
		Eubacterium lentum	-
		Peptostreptococcus micros	-
		Lactobacillus sp. group 1	-
BA	10^4	Selenomonas sputigena	ND[3]
		Fusobacterium nucleatum	
		Lactobacillus sp. group 1	
		Fusobacterium sp. group 1	
		Peptostreptococcus micros	
		Eubacterium sp. group 4	
		Campylobacter sputorum	
		Actinomyces naeslundii	
IN	10^3	Bacteroides ochraceus	100
UJA	10^3	Fusobacterium nucleatum	75
		Bacteroides sp. group 2	25
UJB	10^5	Peptostreptococcus anaerobius	15
		Peptostreptococcus micros	30
		B. melaninogenicus ss. intermedius	30
		Eubacterium sp. group 4	5
		Peptococcus sp. group 1	10
		Fusobacterium sp. group 2	
		Lactobacillus sp. group 3	5
		Selenomonas sputigena	<1
		Fusobacterium nucleatum	5
BN	10^4	Eubacterium sp. group 1	25
		Peptostreptococcus micros	10
		Fusobacterium nucleatum	5
		Eubacterium lentum	10
		B. melaninogenicus ss. asaccharolyticus	15
		Lactobacillus sp. group 3	15
		Campylobacter sputorum	15
		Peptococcus sp. group 1	2
		Fusobacterium sp. group 1	2
		Fusobacterium nucleatum	1
EL	10^3	Fusobacterium nucleatum	100

1) Growth of the isolated strain only after enrichment in broth.

2) Growth of three of the isolated strains only after enrichment in broth.

3) ND, not determined.

pulp chamber may also be greater in teeth with caries or restorations than in teeth with intact crowns.

The bacteria isolated in the present study included a restricted group of species compared to the total flora of the oral cavity. Eubacterium alactolyticum, Propionibacterium acne, Bacteroides melaninogenicus, Campylobacter sputorum, Fusobacterium nucleatum, Veillonella parvula, Arachnia propionica, Peptostreptococcus anaerobius and Peptostreptococcus micros were isolated from some of the teeth. All these species have also been isolated by Wittgow and Sabiston (1975) and all except the last three by Kantz and Henry (1974), which indicates that they frequently occur in the necrotic pulps of teeth with intact pulp chambers (Table V:2). Strains identified as Eubacterium lentum or designated as Eubacterium group 4, Fusobacterium group 2, Peptococcus group 1 and Lactobacillus group 1 and 3 were described only in the present study. These strains could not be successfully subcultured in PYG broth and would have been lost if blood agar had not been used for the initial subcultures. Such strains may also have existed in the teeth investigated by Kantz and Henry (1974) and Wittgow and Sabiston (1975) who reported a significant loss of isolated strains.

The source of bacteria which induce the periapical osteitis in teeth with intact crowns is unknown. One obvious possibility is that the gingival crevice serves as a source. It provides a favourable environment for the growth of anaerobic bacteria (Loesche, 1968) and most of the species that were found in the pulp chambers in the present study have also been isolated from the gingival crevice by Williams et al. (1976)-(Table V:2). Most striking is the recognition of Bacteroides melaninogenicus, Fusobacterium nucleatum and Campylobacter sputorum in the pulp chambers. These are species confined to the gingival crevice and not found in high numbers elsewhere in the oral cavity (Loesche and Gibbons, 1966). They are rarely (Table V:2) recognized in clinical specimens from other sites of the human body (Moore, Cato and Holdeman, 1969). A spreading from the gingival microflora is possible, not necessarily by direct invasion through dentinal tubules but even through the blood stream. Crawford et al. (1974) frequently isolated Bacteroides melaninogenicus and Fusobacterium nucleatum in cubital vene blood sampled after extraction of teeth with periodontitis (Table V:2). Animal studies have shown that bacteria inoculated into the blood may settle in diseased pulp tissue (Robinson and Boling, 1941; Burke and Knighton, 1960; Gier and Mitchell, 1968). These studies together

Table V:2

Occurrence of bacterial strains in samples from dental root canals, gingival crevice, bacteremia and clinical specimens.

	Recovery in per cent of positive samples					
	Dental root canal			Gingival crevice	Bacteremia	Clinical specimens [1]
	Kantz and Henry 1974	Wittgow and Sabiston 1975	Sundqvist 1976	Williams et al. 1976	Crawford et al. 1974	Moore et al. 1969
No. of positive samples	16	32	18	17	23	81
Gram pos. rods						
Actinomyces sp.	31		28	100	26	
Arachnia propionica		3	11		4	
Bifidobacterium				6		4
Clostridium sp.				17		32
Eubacterium alactolyticum	12	12	16			
Eubacterium lentum			16			10
Eubacterium sp.			38			8
Anaerobic Lactobacillus sp.		3	50	23		5
Propionibacterium acne	6	3	5		4	
Propionibacterium sp.				41	8	
Unidentified Gram pos. rods		9		100		2
Gram neg. rods						
Bacteroides corrodens				41	4	
Bacteroides fragilis	6				4	30
Bacteroides oralis		22			22	
Bacteroides ochraceus			11	64		
Bacteroides melaninogenicus	6	6	38	58	60	2
Bacteroides ruminicola		25				
Bacteroides sp.		12	11	94	4	25
Campylobacter sputorum	6	9	33	(11)[2]	(35)[2]	
Fusobacterium nucleatum	25	12	33	94	43	
Fusobacterium sp.	6	12	33		4	5
Selenomonas sp.			11	70		
Unidentified Gram neg. rods		18				2
Gram pos. cocci						
Peptostreptococcus anaerobius		3	27	94	4	5
Peptostreptococcus micros		9	38			
Peptostreptococcus intermedius		3			13	5
Peptococcus magnus					17	10
Peptococcus sp.	6	12	27		8	7
Gram neg. cocci						
Veillonella alcalescens			5	100		1
Veillonella parvula	12	12	5	64	22	1
Facultatively anaerobic bacteria	81	47	27	94	78	66

1)From abdomen, rectum, blood, spinal fluid, pleurum, urogenital, muscle and superficial abcesses.

2)Designated as"Vibrio forms" in the original articles.

with the present one suggest that the gingival crevice should be considered as a source of the bacteria which induce periapical osteitis in teeth with intact crowns.

Most of the infected pulp chambers contained bacteria of several species. However, in each of five of the teeth only one strain could be recovered. It is thus possible that bacteria of one single strain may induce the periapical destruction.

Teeth with non-vital pulps and periapical destruction are usually symptomless but spontaneous acute inflammation in the periapical region may occur. The present study suggests that these exacerbations may be due to the establishment of some specific combination of bacteria in the pulp chamber. Thus in teeth with symptoms of acute inflammation more strains were isolated from the pulp chambers than in the other teeth. Bacteroides melaninogenicus occurred in all seven teeth with acute inflammation but in none of the other teeth. Other bacteria which were found with greater frequency in these teeth were Peptostreptococcus anaerobius, Campylobacter sputorum, Peptococcus group 2 (Peptostreptococcus micros), Peptococcus group 1 and Eubacterium group 1 (Table V:3).

The pathogenicity of Bacteroides melaninogenicus has been demonstrated in animal experiments. Bacteria from the gingival crevice are inoculated subcutaneously. Gangrenous spreading lesions occur from which bacteria could be isolated and used to transmit the infection serially to additional animals (Macdonald et al. 1963; Socransky and Gibbons, 1965). It has been found that transmissable infections develop only with those combinations of bacteria which contain Bacteroides melaninogenicus (Socransky and Gibbons, 1965). Usually three to four strains were necessary in these combinations (Macdonald et al. 1963). The minimal combination was Bacteroides melaninogenicus and an unidentified facultatively anaerobic Gram positive coccobacillus (Socransky and Gibbons, 1965). In conclusion, these studies together with the present results suggest that the acute inflammation in the periapical region is induced by specific combinations of bacterial strains and that the presence of Bacteroides melaninogenicus among these strains is essential for the inducement.

It is a well-known clinical finding that exacerbation sometimes occurs when endodontic therapy is initiated on symptomless teeth with non-vital pulps. One explanation for this finding has been that bacteria from the root canal

Table V:3
Composition of the bacterial flora of 18 teeth with periapical destruction, with and without pain in the periapical region.

	Samples from teeth with pain							Samples from teeth without pain									
	D	G	H	P	AB	UJB	BN	B	C	E	M	U	X	AC	BA	IN	UJA	EL
Fusobacterium group 1, 2			+	+	+	+	+		+						+		+	+
Bacteroides melaninogenicus ss. intermedius/ss. asaccharolyticus	+	+	+	+	+	+	+											
Bacteroides group 2, 3, 5		+						+								+	+	
Peptostreptococcus anaerobius	+	+			+	+			+									
Peptococcus group 2 (Peptostreptococcus micros)	+			+	+	+	+							+	+			
Peptococcus group 1				+	+	+	+		+									
Campylobacter sputorum	+		+	+	+		+								+			
Eubacterium alactolyticum					+				+					+				
Eubacterium group 1			+	+			+											
Eubacterium group 3, 4				+	+	+	+		+					+	+			
Lactobacillus group 1, 2	+	+			+									+	+			
Lactobacillus group 3			+			+	+		+									
Propionibacterium acne													+					
Arachnia propionica				+								+						
Veillonella		+		+														
Selenomonas sputigena						+									+			
Actinomyces	+	+		+									+		+			
Streptococcus mitis		+								+	+							

in these cases have been pushed into the alveolar bone during the instrumentation of the canal (Wittgow and Sabiston, 1975). In the present study acute inflammation in the periapical region occurred in some of the teeth after the treatment. In contrast to teeth without symptoms of acute inflammation they contained a complex flora in which Bacteroides melaninogenicus was present. These results suggest that the presence of a specific combination of bacterial strains in the pulp may be more important for the inducement of the exacerbation than the way in which the instrumentation is performed.

VI. SUMMARY

The present study is a bacteriological investigation of the non-vital pulp. In order to avoid contamination by oral flora teeth with intact crowns were selected. All the teeth could be safely isolated from the oral environment verified in that no bacteria could be isolated in any samples from teeth free from periapical destruction, or in samples from the operation fields or in samples collected during the preparation of the entrance into the pulp chambers. In contrast bacteria were isolated from 18 out of 19 teeth with roentgenological signs of periapical osteitis. Most of these samples contained more than one strain and eight or more strains could be isolated in five teeth. In all 88 bacterial strains were recovered. These results provide strong direct evidence of the etiological role of the isolated bacteria in the development of periapical osteitis.

One striking finding was the dominance of obligately anaerobic bacteria in the samples. Only five of the 88 strains grew in air. One possible explanation for the higher yield of anaerobes in the present study is the careful precautions taken to avoid exposure of the samples to oxygen, from initial sampling and throughout the cultivation. The fact that none of the isolated strains was lost during this investigation is evidence of the favourable cultivation conditions.

For each isolated strain 95 characteristics were determined and included in a numerical taxonomic evaluation in order to estimate the affinities between strains isolated from different teeth. Although there were great differences in the composition of the flora for various teeth the analysis showed that in comparison with oral flora there was a restricted group of bacteria involved in the infection of the pulp chambers. Bacteria of the following genera were frequently found. Fusobacterium, Bacteroides, Eubacterium, Peptococcus, Peptostreptococcus and Campylobacter. However, many strains that were regularly isolated from the root canals have not previously been described.

Acute periapical inflammation defined as tenderness, swelling and exudation occurred in seven patients. In these cases complex anaerobic bacterial flora were present. Bacteroides melaninogenicus was present in samples from all these teeth but in none of the samples from teeth free from these symptoms. Other species which were found in higher frequency in teeth with acute periapical inflammation than in other teeth were Peptostreptococcus anaerobius, Peptostreptococcus micros, Campylobacter sputorum and strains designated as Peptococcus group 1 and Eubacterium group 1.

In conclusion, the present study shows that periapical osteitis is connected with the presence of bacteria in the root canal and it suggests that acute periapical inflammation is generated by certain bacterial species.

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REFERENCES

Aranki, A. and Freter, R. 1972. Use of anaerobic glove boxes for the cultivation of strictly anaerobic bacteria. Amer. J. Clin. Nutr. 25:1329-1334.

Aranki, A., Syed, S.A., Kenney, E.B. and Freter, R. 1969. Isolation of anaerobic bacteria from human gingiva and mouse cecum by means of a simplified glove box procedure. Appl. Microbiol. 17:568-576.

Atterbery, H.R. and Finegold, S.M. 1969. Combined screw-cap and rubber-stopper closure for Hungate tubes (pre-reduced anaerobically sterilized roll tubes and liquid media). Appl. Microbiol. 18:558-561.

Bartels, H.A., Naidorf, I.J. and Blechman, H. 1968. A study of some factors associated with endodontic "flare-ups". Oral Surg. 25:255-261.

Beers, R.J. and Lockhart, W.R. 1962. Experimental methods in computer taxonomy. J. Gen. Microbiol. 28:633-640.

Berg, J.-O. and Nord, C.-E. 1973. A method for isolation of anaerobic bacteria from endodontic specimens. Scand. J. Dent. Res. 81:163-166.

Bergenholtz, G. 1974. Micro-organisms from necrotic pulp of traumatized teeth. Odontol. Revy 25:347-358.

Björkstén, B., Ekstrand, T., Gothefors, L. and Östberg, Y. 1975. The nitroblue tetrazolium (NBT) test and white blood cell count in acute throat infections. Scand. J. Infect. Dis. 7:45-49.

Boone, C.J. and Pine, L. 1968. Rapid method for characterization of Actinomycetes by cell wall composition. Appl. Microbiol. 16:279-284.

Brown, L.R., Jr. and Rudolph, C.E., Jr. 1957. Isolation and identification of microorganisms from unexposed canals of pulp-involved teeth. Oral Surg. 10:1094-1099.

Bryant, M.P. 1974. Selenomonas. Pages 424-426 in R.E. Buchanan and N.E. Gibbons, eds. Bergey's Manual of determinative bacteriology, 8th ed. Williams and Wilkins Company, Baltimore, Md.

Brännström, M. and Nyborg, H. 1971. The presence of bacteria in cavities filled with silicate cement and composite resin materials. Swed. Dent. J. 64:149-155.

Buchanan, R.E. and Gibbons, N.E., eds. 1974. Bergey's Manual of determinative bacteriology. 8th ed. Williams and Wilkins Company, Baltimore, Md.

Burke, G.W., Jr. and Knighton, H.T. 1960. The localization of microorganisms in inflamed dental pulps of rats following bacteremia. J. Dent. Res. 39:205-214.

Caldwell, D.R. and Bryant, M.P. 1966. Medium without rumen fluid for nonselective enumeration and isolation of rumen bacteria. Appl. Microbiol. 14:794-801.

Carlsson, J. 1968. A numerical taxonomic study of human oral streptococci. Odontol. Revy 19:137-160.

Chirnside, I.M. 1957. A bacteriological and histological study of traumatised teeth. N.Z. Dent. J. 53:176-191.

Crawford, J.J., Sconyers, J.R., Moriarty, J.D., King, R.C. and West, J.F. 1974. Bacteremia after tooth extractions studied with the aid of prereduced anaerobically sterilized culture media. Appl. Microbiol. 27:927-932.

Crawford, J.J. and Shankle, R.J. 1961. Application of newer methods to study the importance of root canal and oral microbiota in endodontics. Oral Surg. 14:1109-1123.

Deibel, R.H. and Seeley, H.W., Jr. 1974. Streptococcaceae. Pages 490-509 in R.E. Buchanan and N.E. Gibbons, Eds. Bergey's Manual of determinative bacteriology, 8th ed. Williams and Wilkins Company, Baltimore, Md.

Edwardsson, S. 1974. Bacteriological studies on deep areas of carious dentine. Odontol. Revy 25: Suppl. 32.

Edwardsson, S. and Bowden, G.H. 1974. Identification of Gram positive pleomorphic rods. Pages 47-59 in S. Edwardsson, Bacteriological studies on deep areas of carious dentine. Odontol. Revy 25: Suppl. 32.

Eggen, S. 1974. Röntgenografiske tannmålinger i daglig praksis. Swed. dent. J. 66:10-12.

Elmros, T., Hörstedt, P. and Winblad, B. 1975. Scanning electron microscopic study of virulent and avirulent colonies of Neisseria gonorrhea. Infect. Immun. 12:630-637.

Engström, B. and Frostell, G. 1961. Bacteriological studies of the non-vital pulp in cases with intact pulp cavities. Acta Odontol. Scand. 19:23-39.

Engström, B., Hård af Segerstad, L., Ramström, G. and Frostell, G. 1964. Correlation of positive cultures with the prognosis for root canal treatment. Odontol. Revy 15:257-270.

Fulghum, R.S. 1971. Mobile anaerobe laboratory. Appl. Microbiol. 21:769-770.

Fulghum, R.S., Wiggins, C.B. and Mullaney, T.P. 1973. Pilot study of detecting obligate anaerobic bacteria in necrotic dental pulps. J. Dent. Res. 52:637.

Gerenser, M.A. and Slack, J.M. 1976. Serological identification of Actinomyces using fluorescent antibody techniques. J. Dent. Res. 55 (Spec. issue A): A184-A191.

Gier, R.E. and Mitchell, D.F. 1968. Anachoretic effect of pulpitis. J. Dent. Res. 47:564-570.

Gordon, D.F., Stutman, M. and Loesche, W.J. 1971. Improved isolation of anaerobic bacteria from the gingival crevice area of man. Appl. Microbiol. 21:1046-1050.

Hampp, E.G. 1957. Isolation and identification of spirochetes obtained from unexposed canals of pulp-involved teeth. Oral Surg. 10:1100-1104.

Hampp, E.G. and Mergenhagen, S.E. 1963. Experimental intracutaneous fusobacterial and fusospirochetal infections. J. Infect. Dis. 112-113:84-99.

Hayflick, L. 1965. Tissue cultures and mycoplasmas. Texas Reports on Biology and Medicine 23 (Suppl. 1):285-303.

Holbrook, W.P. and Duerden, B.I. 1974. A comparison of some characteristics of reference strains of Bacteroides oralis with Bacteroides melaninogenicus. Arch. Oral Biol. 19:1231-1235.

Holdeman, L.V. and Moore, W.E.C., eds. 1972. Anaerobe laboratory manual. Virginia Polytechnic Institute and State University, Anaerobe Laboratory, Blacksburg, Va.

Holdeman, L.V. and Moore, W.E.C. 1974. Bacteriodaceae. Pages 384-416 in R.E. Buchanan and N.E. Gibbons, eds. Bergey's Manual of determinative bacteriology, 8th ed. Williams and Wilkins Company, Baltimore, Md.

Holdeman, L.V. and Moore, W.E.C. 1974. New genus Coprococcus, twelve new species, and emended descriptions of four previously described species of bacteria from human feces. Int. J. Syst. Bacteriol. 24:260-277.

Holmberg, K. and Hallander, H.O. 1973. Numerical taxonomy and laboratory identification of Bacterionema matruchotti, Rothia dentocariosa, Actinomyces naeslundii, Actinomyces viscosus and some related bacteria. J. Gen. Microbiol. 76:43-46.

Hungate, R.E. 1950. The anaerobic mesophilic cellulolytic bacteria. Bact. Rev. 14:1-49.

Kafkewitz, D. 1975. Improved growth media for Vibrio succinogenes. Appl. Microbiol. 29:121-122.

Kakehashi, S., Stanley, H.R. and Fitzgerald, R.J. 1965. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg. 20:340-349.

Kantz, W.E. and Henry, C.A. 1974. Isolation and classification of anaerobic bacteria from intact chambers of non-vital teeth in man. Arch. Oral Biol. 19:91-96.

Lamanna, C. and Mallette, M.F. 1965. Page 586 in Basic Bacteriology. Its biological and chemical background. 3rd ed. Williams and Wilkins Company, Baltimore, Md.

Leavitt, J.M., Naidorf, I.J. and Shugaëvsky, P. 1958. The bacterial flora of root canals as disclosed by a culture medium for endodontics. Oral Surg. 11:302-308.

Loesche, W.J. 1968. Some energy sources of Vibrio sputorum. J. Dent. Res. 47:156. (Abstr.)

Loesche, W.J. 1968. Importance of nutrition in gingival crevice microbial ecology. Periodontics 6:245-249.

Loesche, W.J. and Gibbons, R.J. 1966. Influence of nutrition on the ecology and cariogenicity of the oral microflora. Chapter in A.E. Nizel, ed. The Science of Nutrition and its application in Clinical Dentistry. W.B. Saunders Co., Philadelphia, Pa.

Loesche, W.J. Gibbons, R.J. and Socransky, S.S. 1965. Biochemical characteristics of Vibrio sputorum and relationship to Vibrio bubulus and Vibrio fetus. J. Bacteriol. 89:1109-1116

Macdonald, J.B., Gibbons, R.J. and Socransky, S.S. 1960. Bacterial mechanisms in periodontal disease. Ann. N.Y. Acad. Sci. 85:467-478.

Macdonald, J.B., Hare, G.C. and Wood, A.W.S. 1957. The bacteriologic status of the pulp chambers in intact teeth found to be nonvital following trauma. Oral Surg. 10:318-322.

Macdonald, J.B., Socransky, S.S. and Gibbons, R.J. 1963. Aspects of the pathogenesis of mixed anaerobic infections of mucous membranes. J. Dent. Res. 42:529-544.

Macdonald, J.B., Sutton, R.M. and Knoll, M.L. 1954. The production of fusospirochetal infections in guinea pigs with recombined pure cultures. J. Infect. Dis. 95:275-284.

McMinn, M.T. and Crawford, J.J. 1970. Recovery of anaerobic microorganisms from clinical specimens in prereduced media versus recovery by routine clinical laboratory methods. Appl. Microbiol. 19:207-213.

Mejàre, B. 1975. Streptococcus salivarius, Streptococcus sanguis and Streptococcus "mitis" as indicators of contamination of root canal cultures with saliva. Odontol. Revy 26:125-134.

Miller, W.D. 1894. An introduction to the study of the bacterio-pathology of the dental pulp. The Dental Cosmos 36:505-528.

Moore, W.E.C. 1966. Techniques for routine culture of fastidious anaerobes. Int. J. Syst. Bact. 16:173-190.

Moore, W.E.C., Cato, E.P. and Holdeman, L.V. 1969. Anaerobic bacteria of the gastrointestinal flora and their occurrence in clinical infections. J. Infect. Dis. 119:641-649.

Moore, W.E.C. and Holdeman, L.V. 1973. New names and combinations in the genera Bacteroides Castellani and Chalmers, Fusobacterium Knorr, Eubacterium Prevot, Propionibacterium Delwiche, and Lactobacillus Orla-Jensen. Int. J. Syst. Bacteriol. 23:69-74.

Moore, W.E.C. and Holdeman, L.V. 1974. Propionibacteriaceae. Pages 633-657 in R.E. Buchanan and N.E. Gibbons, eds. Bergey's Manual of determinative bacteriology. 8th ed. Williams and Wilkins Company, Baltimore, Md.

Morse, F.W., Jr. and Yates, M.F. 1941. Follow-up studies of root-filled teeth in relation to bacteriologic findings. J. Am. Dent. Assoc. 28:956-971.

Morton, H.E., Smith, P.F., Williams, N.B. and Eickenberg, C.F. 1951. Isolation of Pleuropneumonia-like organisms from human saliva: A newly-detected member of the oral flora. J. Dent. Res. 30:415-422.

Möller, A.J.R. 1966. Microbiological examination of root canals and periapical tissues of human teeth. Akademiförlaget, Göteborg.

Oliet, S. and Sorin, S.M. 1969. Evaluation of clinical results based upon culturing root canals. J. Br. Endod. Soc. 3:3-6.

Palenstein Helderman van, W.H. 1975. Total viable count and differential count of Vibrio (Campylobacter) sputorum, Fusobacterium nucleatum, Selenomonas sputigena, Bacteroides ochraceus and Veillonella in the inflamed and non inflamed human gingival crevice. J. Periodont. Res. 10:294-305.

Palenstein Helderman van, W.H. and Winkler, K.C. 1975. Elective medium for the direct count of Vibrio (Campylobacter), Fusobacteria, Bacteroides, Selenomonas and Veillonella in the gingival crevice flora. J. Periodont. Res. 10:230-241.

Pine, L. and Georg, L.K. 1965. The classification and phylogenetic relationships of the Actinomycetaceae. Int. Bull. Bacteriol. Nomencl. Taxon. 15:143-163.

Pine, L. and Georg, L.K. 1974. Arachnia. Pages 668-669 in R.E. Buchanan and N.E. Gibbons, eds. Bergey's Manual of determinative bacteriology, 8th ed. Williams and Wilkins Company, Baltimore, Md.

Razin, S., Michmann, J., Shimshoni, Z. 1964. The occurrence of Mycoplasma (Pleuropneumonia-like organisms. PPLO.) in the oral cavity of dentulous and edentulous subjects. J. Dent. Res. 43:402-405.

Robinson, H.B.G. and Boling, L.R. 1941. The anachoretic effect in pulpitis. Bacteriologic studies. J. Am. Dent. Assoc. 28:268-282.

Rosebury, T. and Reynolds, J.B. 1964. Continous anaerobiosis for cultivation of spirochetes. Proc. Soc. Exp. Biol. Med. 117:813-815.

Sebald, M. and Véron, M. 1963. Teneur en bases de l'ADN et classification des vibrions. Ann. Inst. Pasteur (Paris) 105:897-910.

Shovelton, D.S. and Sidaway, D.A. 1960. Infection in root canals. Br. Dent. J. 108:115-118.

Smibert, R.M. 1974. Campylobacter. Pages 207-212 in R.E. Buchanan and N.E. Gibbons, eds. Bergey's Manual of determinative bacteriology, 8th ed. Williams and Wilkins Company, Baltimore, Md.

Smibert, R.M. and Holdeman, L.V. 1976. Clinical isolates of anaerobic Gram negative rods with a formate-fumarate energy metabolism: Bacteroides corrodens, Vibrio succinogenes, and unidentified strains. J. Clin Microbiol. in press.

Sneath, P.H.A. 1957 a. Some thoughts on bacterial classification. J. Gen. Microbiol. 17:184-200.

Sneath, P.H.A. 1957 b. The application of computers to taxonomy. J. Gen. Microbiol. 17:201-226.

Sneath, P.H.A. and Johnson, R. 1972. The influence on numerical taxonomic similarities of errors in microbiological tests. J. Gen. Microbiol. 72: 377-392.

Socransky, S.S. and Gibbons, R.J. 1965. Required role of Bacteroides melaninogenicus in mixed anaerobic infections. J. Infect. Dis. 115:247-253.

Socransky, S.S., Macdonald, J.B. and Sawyer, S. 1959. The cultivation of Treponema microdentium as surface colonies. Arch. Oral Biol. 1:171-172.

Sokal, R.R. and Michener, C.D. 1958. A statistical method for evaluating systematic relationships. Kans. Univ. Sci. Bull. 38:1409-1438.

Sommer, R.F. and Crowley, M. 1940. Bacteriologic verification of roentgenographic findings in pulp involved teeth. J. Am. Dent. Assoc. 27:723-734.

Spears, R.W. and Freter, R. 1967. Improved isolation of anaerobic bacteria from the mouse cecum by maintaining continuous strict anaerobiosis. Proc. Soc. Exp. Biol. Med. 124:903-909.

Spellman, A., Socransky, S.S., Hammond, B., Amdur, B. and Krichevsky, M. 1974. Difficulties in classification of oral Gram positive rods. J. Dent. Res. 53(Spec.issue):72 (Abstr.).

Sperry, J. and Wilkins, T. 1976. Suggestions about some "Hard-To-Grow" anaerobes. Anaerobe newsletter No. 3 from Virginia Polytechnic Institute and State University, Anaerobe Laboratory, Blacksburg, Va.

Steers, E., Elwood, L.F. and Graves, B.S. 1959. An inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. Antibiotics and Chemotherpay 9:307-311.

Sundqvist, G. and Carlsson, J. 1974. Lactobacilli of infected dental root canals. Odontol. Revy 25:233-238.

Washington, II, J.A. and Barry, A.L. 1974. Dilution test procedures. Pages 410-417 in E.H. Spaulding and J.P. Truant, eds. Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D.C.

Vêron, M. and Chatelain, R. 1973. Taxonomic study of the genus Campylobacter Sebald and Vêron and designation of the neotype strain for the type species Campylobacter fetus. (Smith and Taylor) Sebald and Vêron. Int. J. Syst. Bacteriol. 23:122-134.

Williams, B.L., Pantalone, R.M. and Sherris, J.C. 1976. Subgingival microflora and periodontitis. J. Periodont. Res. 11:1-18.

Williams, R.A.D., Bowden, G.H., Hardie, J.M. and Shah, H. 1975. Biochemical properties of Bacteroides melaninogenicus subspecies. Int. J. Syst. Bacteriol. 25:298-300.

Winkler, K.C. and van Amerongen, J. 1959. Bacteriologic results from 4,000 root canal cultures. Oral Surg. 12:857-875.

Wittgow, W.C., Jr. and Sabiston, C.B., Jr. 1975. Microorganisms from pulpal chambers of intact teeth with necrotic pulps. J. Endodont. 1:168-171.

Yamada, T. and Carlsson, J. 1975. Regulation of lactate dehydrogenase and change of fermentation products in streptococci. J. Bacteriol. 124:55-61.

Zeldow, B.J. and Ingle, J.I. 1963. Correlation of the positive culture to the prognosis of endodontically treated teeth: a clinical study. J. Am. Dent. Assoc. 66:9-13.

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