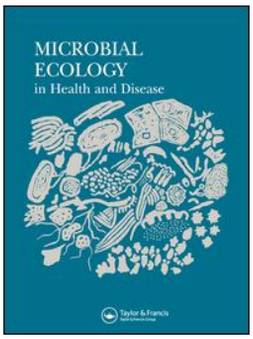

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Salivary Microflora of Geriatric Edentulous Persons Wearing Dentures

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The aim of this study was to investigate the bacterial composition of saliva from five geriatric edentulous persons wearing dentures. Significantly more ($P<0{\cdot}05$) bacteria were recovered after anaerobic incubation (mean $8{\cdot}6 \times 10^8$ c.f.u./ml) than after incubation in air with 30 per cent CO_2 (mean $3{\cdot}8 \times 10^8$ c.f.u./ml). Out of the 151 strains isolated as predominant bacteria, 43 (28 per cent) were obligate anaerobes and 99 (66 per cent) were facultative anaerobes. The majority of the obligate anaerobes and facultative anaerobes isolated were assigned to the genera *Veillonella* and *Streptococcus*, respectively. Strains of *Candida* were detected in 3/5 patients though the viable counts only corresponded to less than 0·001 per cent of the total viable counts. The present study, with the adoption of efficient anaerobic isolation procedures, indicates that many obligate anaerobes can be found in the saliva of edentulous persons wearing complete dentures, and that the bacterial composition of their saliva is similar to that of denture plaque.

KEY WORDS—Anaerobes; Edentulous; Geriatric; Salivary microflora.

INTRODUCTION

The oral microflora has been reported to change quantitatively and qualitatively with age.[3-7] A substantial shift in bacterial composition may occur at the time of losing all the teeth, since teeth provide surfaces for the attachment of organisms best adapted to this niche.[3-7] Some bacteria may be reduced or eliminated by the complete loss of teeth,[6,7] but once a denture is inserted, the bacterial flora may change again.[4,5,22-24,36]

In the oral cavity of edentulous persons, bacteria are found to colonise the tongue, oral mucous membranes and denture surfaces. Since saliva is bacteria-free when secreted, most bacteria in saliva are presumed to have their origin from such oral sites. Some are adapted to the salivary environment, and some contribute to the formation of bacterial deposits at various oral sites, including the denture plaque which may then induce denture stomatitis in some circumstances. Thus, saliva may function as a vehicle for the transfer of bacteria from site to site in the oral cavity.

Comparatively few bacteriological studies have been carried out on saliva of edentulous persons,[4,10,22-25] mostly using selective media for the isolation of specific bacteria, whilst others[7,8,29] have focused on bacteria in the saliva of dentate individuals. The aim of this study was to study the microbial composition of saliva of geriatric edentulous persons, taking particular care to use good isolation methods for strictly anaerobic bacteria.

MATERIALS AND METHODS

Subjects

Edentulous subjects in this study consisted of one male and four females (Table 1), ranging in age from 66 to 71 years. Three of these subjects had been edentulous for more than 20 years, the remaining two for less than 3 years. Loss of teeth occurred as a result of caries (two subjects), periodontitis (two subjects), or a combination of both conditions (one subject). None of the subjects had a history of chronic systemic illness, nor had they taken antibiotics within the 3 mth prior to the bacteriological sampling. In each case, on clinical examination, their complete dentures were in good condition and the oral mucous membranes were clinically healthy.

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Table 1. Some clinical features of the patients in this study

Patient	Age	Sex	Number of years edentulous	Main reasons for losing teeth
1	66	M	1	Caries
2	69	F	>20	Periodontitis
3	70	F	>20	Caries
4	71	F	3	Caries, periodontitis*
5	66	F	>20	Periodontitis

*Posterior teeth were extracted due to caries, and anterior teeth were extracted due to periodontitis.

Samples and isolation of microorganisms

After the complete dentures were removed, resting saliva was collected from the floor of the mouth using a micropipette (Finnpipette, Finland). Sampling was carried out at approximately the same time in the morning. The samples were transported to the laboratory in gas-tight tubes filled with CO_2 within a few minutes and transferred into an anaerobic cabinet (Model AZ-Hard, Hirasawa, Tokyo, Japan) containing 80 per cent nitrogen with 10 per cent H_2 and 10 per cent CO_2. While in the cabinet, 0·5 ml of each sample was mixed with 0·5 ml of sterile 40 mM potassium phosphate 1 mM EDTA buffer (pH 7·0) and dispersed and homogenised with an electric homogeniser (TISSUE-TEAROR, Biospec Products, Bartlesville, OK, USA) and a glass homogeniser. After serial 10-fold dilution with the same buffer, 0·1 ml of each dilution was spread onto surfaces of triplicate brain heart infusion–yeast extract–blood (sheep) agar (BHI-blood agar) plates[12] and incubated anaerobically in the anaerobic cabinet and aerobically in air containing 30 per cent CO_2 at 37°C. Plates, media, buffer solution and experimental instruments were stored under anaerobic conditions for at least 24 h prior to use. All the colonies from each of the suitably diluted samples were isolated in the anaerobic cabinet for further inspection, with the help of a stereomicroscope (×30), if needed. The undiluted homogenised samples (0·1 ml) were also cultured aerobically on selective media for *Candida* (GS media, Eiken, Tokyo, Japan).

Identification of isolates

Microbial genera and species were identified according to the VPI manual,[12,26] supplemented with information relating to the following genera: *Actinomyces*,[31] *Anaerorhabdus*,[34] *Bacteroides*,[14,26] *Eubacterium*,[11,13,28,41] *Enterococcus*,[32] *Fusobacterium*,[27] *Lactobacillus*,[21] *Mitsuokella*,[33] *Prevotella*,[35] *Propionibacterium*,[6] *Stomatococcus*,[3] *Streptococcus*[9,42–44] and *Veillonella*.[30] The following characteristics were examined: gram staining, iodine staining, motility, haemolysis; acid production from adonitol, amygdalin, arabinose, cellobiose, erythritol, esculin, fructose, galactose, glucose, glycogen, inositol, lactose, maltose, mannitol, mannose, melezitose, melibiose, raffinose, rhamnose, ribose, salicin, sorbitol, starch, sucrose, trehalose and xylose; hydrolysis of esculin and starch; liquefaction of gelatin; production of indole; reduction of nitrate; growth in the presence of NaCl (6·5 per cent), lactate and threonine; catalase and urease activities; ammonia liberation from arginine. Volatile fatty acids (C_2 to C_6), acetoin, diacetyl, alcohols (C_2 to C_6), and free acids and/or methyl derivatives of formic, lactic, succinic and phenyl acetic acids, produced in peptone–yeast extract–glucose broth (PYG),[12] were assayed by gas chromatography, as described previously.[1,15,16,20,40] In certain cases, the following characteristics were examined: acid production from inulin; growth in the presence of bile; growth stimulation in the presence of Tween-80, formate–fumarate mixture and horse serum; propionate production from lactate and threonine.

In addition to the tests described above, *Streptococcus* isolates were differentiated by their ability to produce detectable levels of α-L-fucosidase, β-*N*-acetylgalactosaminidase, β-*N*-acetylglucosaminidase and sialidase with 4-methylumbelliferyl-linked fluorogenic substrates.[42–44]

In this study, obligate anaerobes were defined as bacteria which grew only in the anaerobic cabinet, and facultative bacteria as those which also grew in air containing 30 per cent CO_2. It was confirmed at

Table 2. Bacterial recovery from saliva

Sample no.	Anaerobic incubation	Aerobic incubation	Candida
1	$2{\cdot}3 \times 10^7$	$1{\cdot}1 \times 10^7$	ND
2	$7{\cdot}0 \times 10^6$	$5{\cdot}6 \times 10^6$	ND
3	$2{\cdot}8 \times 10^7$	$2{\cdot}2 \times 10^7$	$2{\cdot}0 \times 10^2$
4	$3{\cdot}3 \times 10^9$	$1{\cdot}1 \times 10^9$	$1{\cdot}0 \times 10$
5	$9{\cdot}4 \times 10^8$	$7{\cdot}8 \times 10^8$	$2{\cdot}0 \times 10$
Mean	$8{\cdot}6 \times 10^8$*	$3{\cdot}8 \times 10^8$*	$4{\cdot}6 \times 10$

Results shown as c.f.u./ml.
ND, not detected.
*Significant difference between mean anaerobic and aerobic counts. ($P < 0{\cdot}05$; paired t-test).

least three times that the obligate anaerobes did not grow in air with 30 per cent CO_2. The bacterial genera and species were identified tentatively by 'key characteristics' as described in the VPI manual,[12,26] and more conclusively by combining all the available morphological and biochemical data.

Statistical analysis

Bacterial count data were analysed using the Student paired t-test.

RESULTS

Bacteria, within the range 10^6–10^9 c.f.u./ml, were recovered from the samples of saliva both under anaerobic and aerobic conditions. However, as shown in Table 2, the bacterial counts obtained under anaerobic conditions (mean $8{\cdot}6 \times 10^8$, range $7{\cdot}0 \times 10^6$–$3{\cdot}3 \times 10^9$ c.f.u./ml) were significantly higher (paired t-test, $P < 0{\cdot}05$) than those under aerobic conditions (mean $3{\cdot}8 \times 10^8$, range $5{\cdot}6 \times 10^6$–$1{\cdot}1 \times 10^9$ c.f.u./ml).

All the colonies recovered from each suitably diluted sample were isolated, ranging from 26 to 38 per sample, and a total of 151 isolates were studied further (Table 3). Of these, 103 (68 per cent) were gram-positive bacteria, and consisted of 72 cocci (48 per cent) and 31 rods (20 per cent). Gram-negative bacteria, accounted for 39 (26 per cent) of the isolates, consisting of 26 cocci (17 per cent) and 13 rods (9 per cent).

Obligate anaerobes made up 43 (28 per cent) of the isolates, and 26 (17 per cent) of these were assigned to the genus *Veillonella* which constituted the major portion of isolates in every case (Table 3). Other obligate anaerobes isolated were *Prevotella* (seven isolates; 5 per cent), *Bacteroides* (three isolates; 2 per cent), *Mitsuokella* (one isolate; 0·7 per cent), *Anaerorhabdus* (one isolate; 0·7 per cent), *Fusobacterium* (one isolate; 0·7 per cent), *Actinomyces* (one isolate; 0·7 per cent), *Lactobacillus* (one isolate; 0·7 per cent) and *Eubacterium* (one isolate; 0·7 per cent) as shown in Table 3. The other obligate anaerobe isolated (one isolate; 0·7 per cent) was a gram-negative rod, which could not be identified because of its poor growth and inertness in most biochemical tests. The proportion of obligate anaerobes varied from 3 to 50 per cent of the samples examined (Table 3).

Ninety-nine isolates (66 per cent) were facultative anaerobes, and 51 (34 per cent) were assigned to the genus *Streptococcus* which constituted the major portion of isolates in every case (Table 3). According to their characteristics, they were further identified as *S. parasanguis*, *S. oralis*, *S. salivarius*, *S. mutans*—group, *S. mitis* or *S. intermedius* (Table 4). The second most common bacterial genus among the facultative isolates was *Actinomyces* (20 isolates, 13 per cent). Other genera of facultative anaerobes isolated were *Stomatococcus* (12 isolates, 8 per cent), *Enterococcus* (nine isolates, 6 per cent), *Lactobacillus* (four isolates, 3 per cent) and *Propionibacterium* (three isolates, 2 per cent) as shown in Tables 3 and 4.

Candida was only detected in three out of five subjects, and the viable counts (mean $4{\cdot}6 \times 10$; range $1{\cdot}0 \times 10$–$2{\cdot}0 \times 10^2$ c.f.u./ml) corresponded to 3×10^{-7}–7×10^{-4} per cent of the total viable counts (Table 2).

DISCUSSION

In this study, anaerobic incubation of samples in an anaerobic cabinet yielded a significantly higher bacterial recovery than incubation in air with CO_2 in every case (Table 2). Harding *et al.*[10] who used aerobic dispersion and anaerobic culture of denture plaque material, have also reported that more bacteria were recovered from 60 per cent of samples after anaerobic incubation than after aerobic incubation. However, the bacterial recovery was less than in the present study, suggesting that the stricter anaerobic procedures adopted here helped to preserve the anaerobes, particularly during homogenisation, inoculation and culturing of the samples. The use of similar anaerobic procedures has been shown to improve the recovery of obligate

Table 3. Bacterial isolates from saliva of edentulous patients

	Sample no.					
	1	2	3	4	5	Total
No. of isolates	38	27	33	27	26	151 (100%)
Obligate anaerobes	7 (18%)	1 (3%)	10 (30%)	12 (44%)	13 (50%)	43 (28%)
Veillonella	7	1	7	5	6	26 (17%)
Prevotella	—	—	—	5	2	7 (5%)
Bacteroides	—	—	1	1	1	3 (2%)
Anaerorhabdus	—	—	—	—	1	1 (0·7%)
Mitsuokella	—	—	—	—	1	1 (0·7%)
Fusobacterium	—	—	—	—	1	1 (0·7%)
Actinomyces	—	—	—	1	—	1 (0·7%)
Eubacterium	—	—	—	—	1	1 (0·7%)
Lactobacillus	—	—	1	—	—	1 (0·7%)
Not identified	—	—	1	—	—	1 (0·7%)
Facultative bacteria	28 (73%)	23 (85%)	21 (63%)	15 (55%)	12 (46%)	99 (66%)
Streptococcus	18	11	8	7	7	51 (34%)
Actinomyces	1	9	—	7	3	20 (13%)
Stomatococcus	1	—	8	1	2	12 (8%)
Enterococcus	5	—	4	—	—	9 (6%)
Lactobacillus	—	3	1	—	—	4 (3%)
Propionibacterium	3	—	—	—	—	3 (2%)
Lost	3	3	2	0	1	9 (6%)

anaerobes from oral samples taken from dentate persons.[1,15,18–20,40] Thus, with the adoption of strict anaerobic procedures, such as the anaerobic cabinet, the overwhelming majority (72–92 per cent) of bacteria isolated from dental plaque, periodontal pockets, carious dentine and endodontic lesions of both permanent and deciduous teeth, have been shown to be obligate anaerobes.[1,15,18–20,40]

However, complete loss of teeth may result in a reduction of obligate anaerobes.[36,37] Indeed, in a previous study 49 per cent of the isolates taken from bacterial deposits on complete dentures were found to be obligate anaerobes.[17] In the present study, fewer (28 per cent) obligate anaerobes were isolated from saliva of edentulous persons than has been reported from complete denture plaque, suggesting that the environment of saliva of geriatric edentulous persons may be more aerobic than that of denture plaque.

Gordon and Jong[8] have reported that obligate anaerobes comprise 38 per cent of the salivary flora of dentate adults, even though they used apparently less exacting anaerobic procedures. The discrepancy between this and the results presented here may be ascribed partly to the effects of tooth loss on the salivary microflora and also to the stricter definition of obligate anaerobes employed in this study.

Veillonella and *Streptococcus* constituted the major genera of obligate and facultative anaerobes recovered. This observation was in agreement with previous studies on the salivary flora of edentulous and dentate persons,[4,8,10,25,29] and on the denture plaque of edentulous persons.[4,10,17,39] Amongst the streptococci, *S. parasanguis* and *S. oralis* were found most frequently, together with *S. salivarius*. *S. salivarius* has often been considered to be predominant in the saliva, whereas *S. parasanguis* is a new species which has only recently been described.[2,42–44]

Actinomyces, which has been isolated from denture plaque,[5,17,38,39] was also isolated as one of the predominant bacterial genera. Gram-negative rods, including *Anaerorhabdus*, *Bacteroides*, *Mitsuokella* and *Prevotella* which are newly created genera previously included within the genus *Bacteroides*, often isolated from gingival sulcus,[14,35] were also isolated from saliva in this study. Since these bacteria have not been reported as common components of plaque,[17] it is possible that their main habitat may be the tongue in edentulous persons.

Table 4. Identification of isolates from saliva of edentulous persons (total isolates = 151)

Obligate anaerobes ($n=43$)		Facultatives ($n=99$)	
Veillonella	26	*Streptococcus*	
		S. parasanguis	20
Prevotella		*S. oralis*	16
P. buccae	7	*S. salivarius*	11
		S. mutans-group	2
Bacteroides		*S. intermedius*	1
B. capillosus	1	*S. mitis*	1
B. gracilis	1		
B. ureolyticus-like	1	*Actinomyces*	
		A. odontolyticus	14
Anaeororhabdus		*A. meyeri*	4
A. furcosus	1	*A. israelii*	1
		A. meyeri-like	1
Mitsuokella			
M. multiacidus	1	*Enterrococcus*	
		E. faecalis	5
Fusobacterium		*E. faecium*	4
F. naviforme	1		
		Stomatococcus	
Actinomyces		*S. mucilaginosus*	8
A. odontolyticus	1	*S. mucilaginosus*-like	4
Lactobacillus		*Lactobacillus*	
L. casei subsp. *casei*	1	*L. plantarum*	3
		L. acidophilus	1
Eubacterium			
E. timidum	1	*Propionibacterium*	
		P. acnes	3
Not identified	1		
Lost isolates	9		

In the present study, no or only low numbers of *Candida* were detected (Table 2). Similarly, *Candida* is reported not to be predominant in the saliva[10] and denture plaque[38] of patients with denture-induced stomatitis, or in the denture plaque[17,39] or palatal plaque[5] of edentulous persons.

The present study, using careful anaerobic procedures, revealed that many obligate anaerobes are found in the saliva of edentulous persons wearing dentures and that the bacterial composition of saliva in such persons is quite similar to that reported for denture plaque. Since the bacterial flora of the tongue is another important potential source of bacteria which may be spread to other sites in the mouths of edentulous persons, a further study on the bacteria colonising the tongue is now in progress.

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