APPLIED MICROBIOLOGY, Feb. 1968, p. 428-429

Vol. 16, No. 2
Printed in U.S.A.

Indigenous Flora from Human Saliva

D. F. GORDON, JR., AND B. B. JONG
Forsyth Dental Center, Boston, Massachusetts 02115

Received for publication 9 October 1967

In previous studies of saliva, the various microbial types have been enumerated by plating diluted samples directly onto selective media (R. L. Richardson and M. Jones, J. Dental Res. **37**:697, 1958; S. L. Handelman and J. R. Mills, J. Dental Res. **44**:1343, 1965; R. H. Bloom and L. R. Brown, Jr., Oral Surg. Oral Med. Oral Pathol. **17**:658, 1964). Although many samples can be examined in this manner, the most numerous organisms present may not necessarily be identified. The present study was initiated in an attempt to isolate all those organisms present in high dilutions of saliva cultivable on blood-agar under anaerobic conditions. Anaerobic incubation was used, since obligate aerobes are rare among the indigenous oral flora (S. S. Socransky et al., Arch. Oral Biol. **8**:275, 1963; W. J. Loesche and R. J. Gibbons, p. 307, *in* A. E. Nizel [ed.], *The Science of Nutrition and its Application in Clinical Dentistry*, W. B. Saunders Co., Philadelphia, 1966).

Unstimulated saliva was collected from six adults (age, 21 to 31 years) in sterile test tubes at least 2 hr after they ate breakfast. The procedures described by R. J. Gibbons et al. (Arch. Oral Biol. **8**:281, 1963) were followed in dispersing the samples and in plating high dilutions on Heart Infusion Agar (Difco) supplemented with 10% horse blood. Plates were incubated for 5 days at 35 C in Brewer jars containing 95% H_2 and 5% CO_2. A 10 × dissecting microscope was used to aid in the isolation of all organisms from plates containing approximately 50 to 100 colonies. A total of 373 colonies were isolated and partially characterized by standard procedures (Society of American Bacteriologists, *Manual of Microbiological Methods*, McGraw Hill Book Co., Inc., New York, 1957) and distributed into eight microbial groups (Table 1).

The gram-positive facultative cocci represented the largest single group. Streptococci alone comprised 41.0% of the saliva isolates. All 151 strains of streptococci fermented glucose and did not reduce nitrate or produce catalase. Five isolates presumably were enterococci as they grew in 6.5% sodium chloride broth, reduced 0.1% methylene blue milk, and hydrolyzed starch. Seventeen strains produced mucoid colonies on Mitis Salivarius Agar (Difco) and were considered to be strains of *Streptococcus salivarius*. The remaining strains were designated *Streptococcus mitis*. Fifteen strains resembled staphylococci in morphology but were catalase-variable and failed to grow on Chapman Stone Medium (BBL). Most of these strains resembled *Staphylococcus salivarius* (D. F. Gordon, J. Bacteriol. **94**:1281, 1967). Five organisms in this group were lost before they could be characterized.

Of the 65 strains of gram-negative anaerobic cocci, 58 apparently were *Veillonella* as they grew on Rogosa lactate agar (M. Rogosa et al., J. Bacteriol. **76**:455, 1958) and did not utilize glucose, but reduced nitrate. In addition, 10 strains produced a catalase-like reaction which apparently is the result of a nonheme peroxide decomposing enzyme (M. Rogosa, J. Bacteriol. **87**:162, 1964). The gram-positive anaerobic cocci are probably members of the genera *Peptostreptococcus* or *Peptococcus*. None of the 47 isolates was catalase-positive, and variability was exhibited in glucose utilization, starch hydrolysis, and nitrate reduction.

The gram-positive facultative and anaerobic rods (42 and 19 strains, respectively) failed to grow on Rogosa SL Medium (Difco), and therefore none of these strains appeared to be lactobacilli. Only 10 strains produced catalase; these may be members of the genera *Corynebacterium* or *Propionibacterium*. The catalase-negative anaerobes (15 strains) were presumably *Actinomyces* (E. G. Rasmussen, R. J. Gibbons, and S. S. Socransky, Arch. Oral Biol. **11**:573, 1966). The majority of the gram-positive rods represented a poorly defined group that are generally referred to as diphtheroids.

Only one of the 17 strains of gram-negative anaerobic rods possessed the tapered ends characteristic of *Fusobacterium*. Eight strains were considered to be *Vibrio sputorum* as they reduced nitrate but did not utilize glucose. *Bacterioides* species appeared to comprise the remaining isolates. The gram-negative facultative rods (eight strains) and gram-negative facultative cocci (four strains) could not be identified on the basis of the limited tests performed.

Presumably, microorganisms are present in

TABLE 1. *Distribution of microorganisms isolated from saliva*

Individual	No. of isolates	Gram-positive facultative cocci	Gram-negative anaerobic cocci	Gram-positive anaerobic cocci	Gram-positive facultative rods	Gram-negative anaerobic rods	Gram-positive anaerobic rods	Gram-negative facultative rods	Gram-negative facultative cocci
1	57	40.4[a]	17.5	5.3	17.5	14.0	0.0	5.3	0.0
2	85	35.2	32.9	8.2	12.9	1.2	8.2	1.2	0.0
3	55	49.1	1.8	18.2	18.2	5.5	5.5	1.8	0.0
4	76	60.5	14.5	11.8	1.3	2.6	5.3	1.3	2.6
5	45	55.5	8.9	8.9	13.3	2.2	4.4	2.2	4.4
6	55	36.4	20.0	25.5	7.3	3.6	5.5	1.8	0.0
Total and mean per cent	373	46.2	15.9	13.0	11.8	4.8	4.8	2.3	1.2

[a] Percentage each microbial group represented of the isolates from each individual.

saliva as a result of dislodging them from various sites in the oral cavity, e.g., the gingival crevice area, dental plaque, or the tongue. Although it is difficult to assess the exact source of these organisms, the distribution of the tongue microflora appears to reflect most closely that found in saliva (D. F. Gordon and R. J. Gibbons, Arch. Oral Biol. **11**:627, 1966).

This investigation was supported by research grant DE-01471 from the National Institute of Dental Research, and by a grant from Colgate-Palmolive Co.