DISTRIBUTION OF NEISSERIA, ROTHIA AND STREPTOCOCCI IN EARLY STAGES OF DENTAL PLAQUE

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ABSTRACT

Distribution of Neisseria, Rothia and streptococci in the early stages of dental plaque was studied on 6 adult subjects. The dental plaque was collected from the surface of the upper incisor at 0, 1, 4 and 12 hours after thoroughly brushing the teeth.

Although marked individual differences were observed, Streptococcus was the most predominant and Rothia was also nearly the same as Streptococcus. Neisseria was less in number. The mean distribution of Neisseria was 9.2% (at 0 hour), 11.3% (at 1 hour), 1.8% (at 4 hours) and 3.5% (at 12 hours) and showed a decreasing trend with the passing of time. Streptococci and Rothia showed approximately the same values in all plaque samples.

The distribution of the streptococcal species was studied. S. facalis could not be found in all of the plaque samples. S. mitieri and S. salivarius were infrequently found and the latter was considered to be the contaminant of saliva. S. mutans was also of the minor group in the early surface plaque. S. mitior and S. sanguis were the most predominant species in all stages of the plaque.

With regard to the growth rate of the 3 genera, the streptococci were the highest, reaching the stationary phase in 12 hours. Rothia showed a longer lag-phase but the final growth rate was nearly the same as the streptococci. Neisseria showed the slowest growth, the lag-phase continuing up to 20 hours.

The process of initiation and maturation of the plaque were discussed in relation to the growth rate and adhesion capacity.

INTRODUCTION

Many authors reported that streptococci, Neisseria and Rothia were the most predominant organisms in the early stage of dental plaque[1-4]. Ichinokawa[5] suggested that the amylolactic-like polysaccharide-producing Neisseria initially was adsorbed on to the tooth and thereafter the streptococci colonized.

We reported in the previous study that N. sicca and N. subflava were the most predominant species in Neisseria and had the capacity of being adsorbed on to the glass surface in vitro (Horikawa et al.)[6]. However, it was considered that Neisseria was not the predominant organism in the early plaque.

In this experiment, the distribution of streptococci, Rothia and Neisseria in the early stage of the plaque was examined.

MATERIALS AND METHODS

Dental plaque. The dental plaque of the early stage was collected from 6 adult subjects who had clinically healthy incisor teeth and gingiva. The upper incisor teeth were thoroughly cleaned by brushing, and
the plaque were collected from the labial surface at 0, 1, 4 and 12 hours after brushing by means of sterilized small tampons. Salivary contamination was avoided as much as possible. The plaque sample was put into 5 ml of sterilized physiologic saline supplemented with 0.02% yeast extract and stirred vigorously. The plaque solution was immediately diluted with tenfold dilution, and 0.1 ml of the appropriate dilution was inoculated onto the isolating media by a bent glass rod.

Media and isolation of organisms. 1) Neisseria: BTB-lactose agar (Eiken) was used. The inoculum was incubated at 37°C for 48 hours. Lactose-fermenting and oxidase-positive colonies were counted as Neisseria. (This was confirmed in the previous report.)

2) Rothia: Brighton’s medium9) was used, composed of Na-glucuronate 1%, Tryptone (Oxoid) 0.1%, Lab crevo powder (Oxoid) 0.5%, agar 1.5%, and NaF (25 mg/ml) 0.5%. After incubation aerobically for 48 hours at 37°C, 30 strains were isolated at random. The gram-positive filamentous organisms were identified as Rothia. When gram-positive cocci were found in the isolates, the organisms were inoculated into the brain heart infusion (BHI) broth (Difco), and those showing filamentous forms were also identified as Rothia. Number of Rothia was determined by multiplying percentage of Rothia in the 30 isolated strains to the total number of colonies on the plate.

3) Streptococci: Mitis-Salivarius agar (MS) (Difco) was used for obtaining the total number of streptococci. S. mutans were calculated on Gold’s medium10). Streptococci were incubated anaerobically (N2 88%, CO2 10%, H2 7%) for 48 hours at 37°C. Eighty colonies were isolated at random from each plaque sample on the MS agar by using a stereomicroscope and purified on the BHI blood agar.

Biological characterization of streptococci. Heart infusion broth was used for the basal medium throughout the test.

1) Fermentation of mannitol: Mannitol 1%, glucose 0.05% and methyl red 0.02% were added to the basal medium. The fermentation reaction was observed for 10 days.

2) Hydrolysis of aesculin: Glucose 0.05%, aesculin 0.1% and ferric citrate 0.05% were added to the basal medium. Blackening of the medium after 10 days of incubation was determined as a positive reaction.

3) Dextran and levan production: 5% sucrose-supplemented heart infusion (HI) broth was used. After the incubation of streptococci for 48 hours, the supernatant solution was obtained and diluted 5 times with distilled water. One and two-tenths ml of ethanol were added to 1 ml of diluted supernatant and the precipitation was observed. For levan production, another 1.5 ml of ethanol were added.

4) H2O2 production: Whittenberg’s method11) was used, and sterilized horse defibrinated blood was used for the detection. H2O2 production was detected by the blackening of the medium.

Growth rate of the organisms. Two representative strains were selected from the isolated S. mitis, S. sanguis, S. milleri, Rothia and Neisseria, respectively. Each strain was subcultured twice in the BHI broth for 24 hours, and 2 ml of the culture were inoculated in 200 ml of the BHI broth. Rothia and Neisseria were incubated aerobically and streptococci anaerobically. Bacterial growth at an appropriate incubation time was detected by turbidity by using a spectrophotometer (Shimazu UV-200) at a wave length of 600 nm.
Adsorption of the organisms on to glass surface. Five strains were selected from each isolated species and incubated in 5% sucrose-added HI broth for 24 hours and the adsorption on to the tube wall was observed.

RESULTS

Lactose-non-fermenting and oxidase-producing organisms on BTB-lactose agar were all identified as Neisseria. Almost all of the organisms grown on Beighton's medium were gram-positive and filamentous and were considered as Rothia. Very few organisms other than streptococci were found on the MS agar and were considered to be negligible.

Distribution of streptococci, Rothia and Neisseria. Relative distribution of the 3 genera are shown in Figure 1.

Neisseria were less than 10% in all cases except in the 1-hour samples of MF (16%) and NT (41%). The distribution of Rothia was between 16 and 64% with some exceptions, being somewhat less than the streptococci. The mean value of the distribution of the 3 genera at various periods is shown in Figure 2. Since marked individual differences were observed in the distribution of the organisms, the plaque-forming process within 12 hours was not distinct. Among the 3 genera, Neisseria showed a decreasing trend with the passing of time. In the early stage of the plaque, streptococci and Rothia showed almost the same value, but in the 12-hour plaque, streptococci were more predominant than Rothia.

Distribution of streptococcal species. Approximately 80 strains of streptococci were isolated from each plaque sample except at 0 hour, 1,700 strains being isolated in all. Differentiation of the streptococci were carried out by Hardie's short set\(^{12}\) and Bergey's manual\(^{13}\).

S. faecalis was not detected.

A small number of S. salivarius was found when contamination of saliva occurred on the tooth surface. The individual distribution of the streptococcal species is shown
in Figure 3. S. mutans was detected only in the 1 and 4-hom plaque of 2 subjects (NF and NT). S. mitior and S. sanguis were found in all samples. S. mitior was the most predominant species in the early stage of the plaque, 1,078 of 1,700 strains (63.9%) being identified as this species. S. sanguis was found in 527 of 1,700 strains (31.3%). Species transition in the course of time could not be detected because of the
marked difference in the individuals.

*Adsorbance on to glass surface.* Five strains of each species were tested for the capacity of being adsorbed on to the glass surface in vitro. Five strains of S. mutans, 3 of Rothia, 2 of N. sicca and 1 of N. subflava showed this capacity but none with S. miller, S. sanguis or S. mitior.

*Growth rate.* As shown in Figure 5, S. mitior showed the highest growth rate, followed by S. sanguis and S. miller.

Rothia showed a long lag-phase, and before 12 hours of culture the growth became log-phase. But the growth rate was approximately the same as the streptococci. In contrast, Neisseria showed a very low growth rate and the lag-phase continued for more than 12 hours.

**Discussion**

In this experiment, the incidence of the transition of the microbial composition in the early stages of the dental plaque was studied. Plaque samples were collected from the upper incisor surface at 0, 1, 4 and 12 hours after cleaning the teeth. Brushing was used for cleaning and it was ascertained that there was no detectable plaque on the surface. The relative number of streptococci, Rothia and Neisseria in the plaques were detected each time. In the case of the one-hour plaque, Neisseria was detected in approximately 10% of the cases and Rothia and streptococci were detected in a little more than 40% of the
Fig. 3c

Fig. 3. Distribution of Streptococcal Species.
reported that gram-positive facultative cocci were detected in 46%, gram-positive anaerobic cocci in 13.0%, gram-positive facultative rods in 11.8% and gram-negative facultative cocci in 1.2%. On the other hand, Gibbons et al.10 reported that the distribution of bacteria in the gingival debris was gram-positive facultative cocci 28.8%, gram-positive anaerobic cocci 10.7%, gram-positive facultative rods 5.8% and gram-negative facultative cocci 0.4%. In comparison to these reports, Neisseria and Rothia were more predominant in the early stage of the plaque. Some strains of Rothia and Neisseria were shown to have the capacity of being adsorbed on to the glass surface in vitro. However, S. sanguis and S. mitior did not, although some of them produced dextran from sucrose.

From these results, the following speculation may be made. Neisseria and Rothia from the environment may initially be adsorbed onto the smooth tooth surface and, thereafter, streptococci may be adsorbed by agglutination or by some modification of the tooth surface which occurred by the adsorbance of Neisseria or Rothia. Since streptococci are the most predominant in the saliva and on the surface of the mucous membrane, they are predominant in the early state of plaque formation. The reason for the decrease of Neisseria in 4 hours may be related to its slow growth rate or to the unknown environmental conditions. According to the proliferation of the streptococci, the oxidation-reduction potential may decrease and an anaerobic condition may occur. Since Neisseria and Rothia prefer an aerobic condition rather than an anaerobic, such a condition is not suitable for them. This may result in the trend of the decrease of the Rothia and Neisseria in the course of the maturation of the dental plaque. Ritz25 reported that
the Rothia in the 24-hour plaque was one-sixth of the streptococci.

During the experiment, many organisms other than the 3 genera described in this report were detected. Studies on such organisms are now in progress.

REFERENCES


