

The presence of methanobacteria in human subgingival plaque

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Abstract. Through a procedure of setting up a strictly anaerobic chain from sampling subgingival plaque to incubation in an anaerobic cabinet ($O_2 < 10$ ppm), it was possible to, for the first time, isolate methanobacteria from human subgingival plaque.

Key words: Subgingival plaque – methanobacteria – anaerobiosis.

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It is well-known that different species of micro-organisms are present in the subgingival plaque, in both healthy and pathological conditions (Socransky 1977). Data given in the literature indicate that certain microbial flora may be compatible with a state of periodontal health (prevalent aerobic microflora), while different flora (mostly anaerobic microflora) are associated with varying degrees of periodontal disease (Listgarten & Helldén 1978). It may be assumed that in the oral cavity, the presence in the subgingival plaque of an anaerobic mixed microflora could lead to the methanogenesis process, similar to that which occurs in the mammalian bowel, rumen and anaerobic digestors (Zeikus 1977, Sorlini et al. 1983). The methanogenesis process is the result of the activity of 3 trophic groups of micro-organisms: hydrolytic, acetogenic and methanogenic bacteria. The latter are very strictly anaerobic micro-organisms able to utilize, for production of CH_4 , the products obtained from fermentation performed by different micro-organisms present in the anaerobic ecosystem, such as acetate, formate, methanol, CO_2 and H_2 .

There are no reports on the presence of methanobacteria in human subgingival plaque. However, Kemp et al. (1983) have reported the presence of methanogenic bacteria in monkey dental plaque.

In this report, we demonstrate the presence of methanobacteria in human subgingival plaque.

Patient selection and method of sampling of subgingival plaque were carried out according to the criteria adopted by

Socransky et al. (1963). 10 adults, male and female, ranging in age from 20 to 35 years, were examined after 24 h without oral hygiene. A pool of subgingival plaque was collected from the gingival crevice area of each subject by means of sterile curettes and placed immediately in vials containing pre-reduced Aranki medium (Aranki et al. 1969) maintained in strictly anaerobic conditions in Anapak Jars (Scott Laboratories, Fiskeville, Rhode Island) until use. No later than 1 h after sampling, the specimens were placed in an anaerobic cabinet and the contents of each vial (containing plaque from a single patient) were directly inoculated into a 30 ml vial containing 10 ml of pre-reduced Balch I medium (Balch et al. 1979) and 20 ml of

the atmosphere present in the anaerobic cabinet. The atmosphere present in the vials was then replaced for with CO_2 and H_2 (80%, 20%) and the cultures incubated at $35^\circ C$ for 10–20 days. The cabinet used was the Forma Scientific Anaerobic Glove Cabinet, model 1024 (Forma Scientific, Marietta, Ohio), modified by us (Brusa & Ferrari 1985) to make it possible to work constantly in an atmosphere of N_2 , H_2 and CO_2 (85%, 10%, 5%) at an oxygen pressure of less than 10 ppm.

After 10 and 20 days of incubation, the presence of methane was detected by gas-chromatographic analysis using a Dani model 3200 HWD gas-chromatograph equipped with a column (1 m \times 4 mm) packed with a molecular

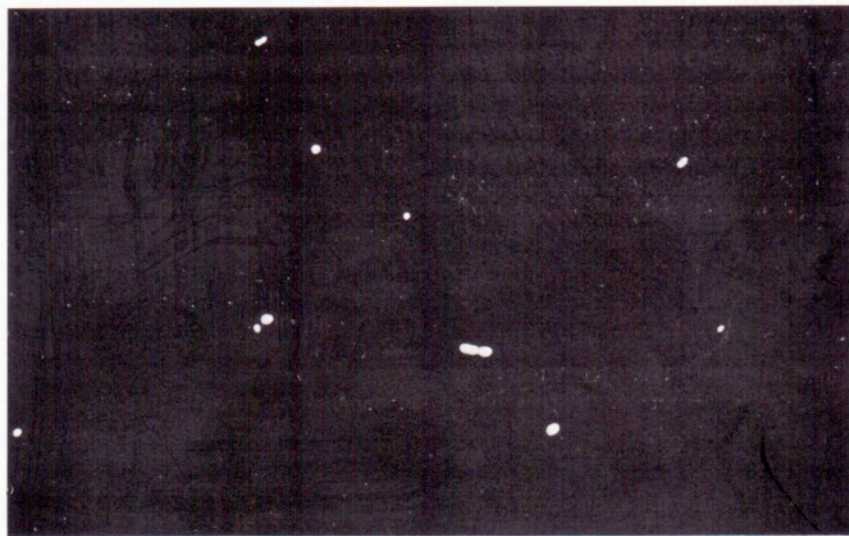


Fig. 1. Methanobacteria present in enrichment culture from human subgingival plaque. Photomicrograph taken with epi-illumination at 420 nm ($\times 1400$).

sieve and thermal conductivity detector. Analysis conditions were: injector and oven, 50°C; carrier gas, H₂; flow rate, 20 ml/min. Samples of cultures were also observed using a microscope equipped for epifluorescence with an H 436 filter set and an LP 470 barrier filter (Zeiss); the presence of methanogenic bacteria was shown by the characteristic fluorescence specific to the F 420 factor.

Methanobacteria were observed in 3 of the 10 pools of subgingival plaque tested. After 10 days of incubation, the presence of methanobacteria was already revealed by a check of methane production and specific fluorescence, according to Zeikus (1977), Doddema & Vogels (1978) and Vogels et al. (1980). The results were confirmed by a 2nd series of tests performed after 20 days of incubation. 1 ml from each of the 3 cultures in which methanobacteria were observed, was transferred to Balch 1 medium supplemented with clindamycin (1.5 µg/ml) and cephalothin (5 µg/ml) (Godsy 1980). After 20 days of incubation, selected cultures were obtained. These cultures were streaked on agarized Balch 1 medium (Miller & Wolin 1982). Thus, after incubation, the development of colonies with fluorescence at 420 nm was obtained. All the colonies had the same morphological characteristics: they were circular with complete margins, translucent, convex, and with a pale-yellow pigmentation.

Strictly anaerobic Gram-positive bacteria, showing the same morphology, were isolated in pure cultures from the colonies. They appeared as extremely fluorescent short rods (0.5 × 0.6–0.8 µm) occurring singly, in pairs, or in short chains (Fig. 1). The pure cultures, grown in Balch 1 medium, were able to produce methane after 10 days of incubation. From general characteristics, the methanobacteria recovered from the 3 subjects were classified as belonging to the *Methanobrevibacter* genus (Whitman 1985; Balch et al. 1979).

Conclusions

The method we used permitted us for the first time to detect the presence of

methanobacteria in human subgingival plaque. This is a preliminary survey; further studies are in progress, in particular to identify the isolated methanobacteria and to verify possible correlation between different degrees of pathologic conditions and the presence of these micro-organisms.

Zusammenfassung

Das Vorkommen von Methanobakterien in der subgingivalen Plaque des Menschen.

Durch das Ausarbeiten einer strikt anaeroben Kette von Arbeitsmomenten – von der

Stichprobenentnahme aus der subgingivalen Plaque bis zur Inkubation in einer anaeroben Kammer (O₂ < 10 ppm) – war es möglich, zum ersten Male Methanobakterien aus der subgingivalen Plaque des Menschen zu isolieren.

Résumé

Présence de méthanobactéries dans la plaque sous-gingivale humaine.

Une méthode conçue pour assurer une chaîne rigoureusement anaérobie, depuis le prélèvement d'échantillons de plaque sous-gingivale, jusqu'à l'incubation dans une cabine d'anaérobiose (O₂ < 10 ppm), nous a permis d'isoler pour la première fois des méthanobactéries dans la plaque sous-gingivale humaine.

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