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# Colonization of the Cementum Surface of Teeth by Oral Gram-Negative Bacteria

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By using in vitro assays, a group of related, filamentous gram-negative bacteria isolated from subgingival plaque deposits of patients with periodontal disease were found to colonize intact teeth. Tentatively identified as members of the genus *Cytophaga*, these isolates exhibited a preference for colonizing the cementum surface of the root. Examination of intact teeth after several weeks of colonization revealed that the root substructure had been extensively demineralized.

The oral cavity is host to a large and diversified population of microorganisms (4). Recent studies have demonstrated that some members of this population adhere to human tissues in a more or less specific fashion. For example, *Streptococcus mitis* attaches to epithelial cells of the buccal mucosa and teeth (8), whereas *Streptococcus salivarius* and *Streptococcus pyogenes* attach to epithelial cells lining the dorsum of the tongue (5) and throat (3), respectively. *Streptococcus sanguis* (15) and *Streptococcus mutans* (4) colonize tooth surfaces presumably by initially attaching to a layer of salivary glycoproteins that coat the teeth or by synthesizing an adhesive glucan from dietary carbohydrates.

In addition to providing themselves with a specific econiche, those bacteria involved in the initial attachment to various tissues also serve as a potential substratum for secondary colonizations (2). Thus, electron microscopic studies (10) of dental plaque from both sub- and supragingival deposits reveal that a variety of morphologically diverse microorganisms can interact directly (cell-to-cell contact) or indirectly (via a matrix) to form, ultimately, a complex microbial community.

A study by Listgarten (9) describing the microbial composition of subgingival plaque obtained from periodontitis patients was of specific interest to us because electron micrographs revealed the presence of filamentous bacteria on or near the cementum surface of the tooth root. These bacteria were morphologically similar to a group of microorganisms that we had recently isolated from the plaque of patients with advanced periodontal disease. As part of a biochemical and genetic characterization of these filamentous gram-negative bacteria, their ability to colonize teeth was also tested. This report describes the preferential attachment of these organisms to the cementum surface of the root and our initial attempts to determine the means by which they adhere to that surface.

(A preliminary report of this work was presented at the annual meeting of the International Association of Dental Research 1979, New Orleans, La., abstract 638, p. 252.)

## MATERIALS AND METHODS

**Microorganisms.** The strains used in the studies reported here, DR2001, DR2002 and DR2021, were drawn from a collection of over 20 strains of filamentous, gram-negative bacteria isolated from subgingival plaque samples of patients with destructive periodontitis. The group as a whole, and the three selected strains specifically, exhibited limited saccharolytic capabilities; of 22 sugars tested, only glucose, sucrose, maltose, and mannose were fermented with concomitant acid production. Succinate and acetate were the major end products of glucose fermentation and appeared in a ratio of roughly 5 to 1. The three strains exhibited gliding motility whether harvested from agar-based solid media or liquid cultures. Their guanosine-plus-cytosine ratios are similar, varying only between 39 and 40% (J. Johnson, private communication). Finally, the organisms were capable of growing either aerobically or anaerobically and exhibited no $CO_2$ requirement in air.

Morphologically and biochemically, these bacterial strains resemble microorganisms assigned to the newly proposed genus *Capnocytophaga* (6, 14) as well as the facultatively anaerobic members of the family *Cytophaga* (7). Since our strains lack the $CO_2$ requirement characteristic of the genus *Capnocytophaga*, textual references to them are made as *Cytophaga* sp., recognizing the fact that these bacteria may belong to the genus *Flexibacter* or may be variants of *Capnocytophaga* sp.

**Adherence assays.** Stock cultures were main-

tained in Schaedler broth (BBL Microbiology Systems, Cockeysville, Md.). After 72 h of incubation at 37°C in air or gas packs containing $H_2$ and $CO_2$ (GasPak; BBL Microbiology Systems), stock cultures were maintained at ambient temperatures and transferred biweekly or weekly. Intact tooth binding studies were carried out using healthy extracted and cleaned teeth mounted on steel-Nichrome (Hoskins Manufacturing Co., Detroit, Mich.) wires by the technique of McCabe et al. (12). After attachment to their wire supports, the teeth were sterilized by autoclaving or exposure to ethylene oxide and suspended in test tubes containing Schaedler broth supplemented with an appropriate sugar, 0.005 M $K_2HPO_4$, and 0.03 M NaCl. Tubes were then inoculated with one of the strains of *Cytophaga* sp. The teeth were incubated at 37°C in GasPaks for a period of 21 days; spent medium was replaced with fresh broth at 3-day intervals. At the end of the incubation period, plaque accumulation was graded by the method of McCabe et al. (12). To determine whether (i) sucrose was essential for the attachment of cells to teeth and (ii) dextran was present in the plaque matrix, the intact tooth assay was modified as follows. Trace amounts of sucrose were removed from the incubation medium by adjusting the pH to 4.5 and adding 2 mg of invertase per 100 ml of medium (2.5 IU per ml) after a 2-h incubation at 50°C, the medium was autoclaved and sterile glucose was added to a final concentration of 0.5%. The presence of dextran in plaque was determined qualitatively by the method of McCabe and Donkersloot (11). Two-week-old wire-bound plaques were treated with commercial endodextranase for 24 h at 37°C and compared with plaques produced by *S. mutans* 6715-13 wild type treated in the same fashion.

## RESULTS

When the in vitro plaque assay of McCabe et al. (12) was used, strains DR2001, DR2002, and DR2021 colonized the root surfaces of teeth mounted on wire after 3 to 4 days of incubation. Further incubation resulted in the development of a heavy, tenacious, amber-colored bacterial mass that exhibited a relatively high degree of specificity for attachment to the cementum surface of the root (Fig. 1A and 2). On prolonged incubation (greater than 3 weeks), the bacterial mass occasionally increased to a point where its weight distorted the bacterial deposit and caused it to cover the enamel surface. However, the adhesiveness of the plaque to the enamel surface in these instances was as weak or weaker than its affinity for the wire support. Inverting the tooth prevented the plaque from growing over the enamel surface, and bacterial growth on the crown only occurred in the crevices and wire-crown interstices (Fig. 2A). When the tooth was sectioned longitudinally and incubated in the same fashion as intact teeth, the *Cytophaga* strains quickly colonized both the root surface and the entire exposed portion of the tooth's interior (Fig. 2B). Experiments identical to those shown in Fig. 1A and 2 were repeated at least four times with each strain and yielded substantially identical results (Fig. 3A and C). Since the teeth were randomly selected specimens from a variety of human sources and colonization also occurred in Trypticase soy broth (BBL Microbiology Systems) supplemented with 0.5% glucose or sucrose (Fig. 3B), the phenomenon does not appear to be restricted to a specific set of growth conditions.

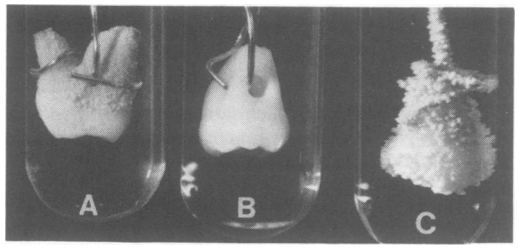


FIG. 1. *Comparative modes of colonization of two oral bacteria. Cytophaga sp. strain DR2001 after 3 weeks of incubation (A); control tooth (B); S. mutans strain 6715-13 wild type after 2 weeks of incubation (C). Note specificity of attachment of Cytophaga sp. to the root surface.*

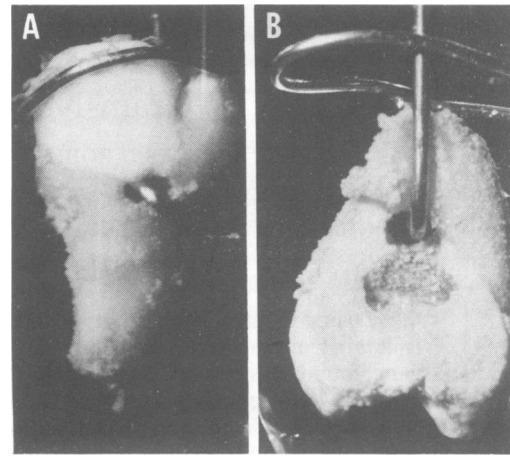


FIG. 2. *Specificity of attachment exhibited by Cytophaga sp. strain DR2001. (A) Inverted tooth with colonization restricted to root area and narrow interstices between mounting wire and tooth. (B) Adherence of Cytophaga sp. DR2001 to exposed dentin surface of a sectioned tooth.*

The pattern and specificity of *Cytophaga* sp. colonization contrast sharply with those of *S. mutans*; the latter adhere to the surfaces of the crown, root, and support wire with the same apparent tenacity (Fig. 1C). Furthermore, unlike *S. mutans*, glucose may be substituted for sucrose in the incubation medium of the gram-negative isolates without affecting their ability to colonize the root surface. To completely di-

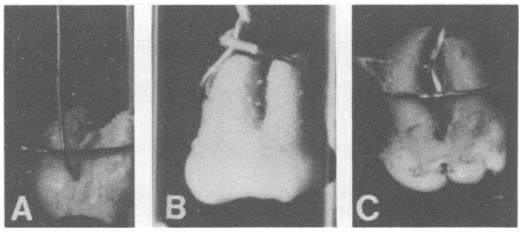


FIG. 3. *Attachment of other strains of Cytophaga sp. to human teeth. (A) Strain DR2002 incubated in Schaedler broth. (B) Strain DR2002 incubated in Trypticase soy broth. (C) Strain DR2021 incubated in Schaedler broth.*

vest the requirement for sucrose from the colonization process, the incubation medium was treated with filter-sterilized invertase at a concentration of 20 μg/ml (2.5 IU/ml) for 2 h before autoclaving. This treatment had no effect on colonization by the *Cytophaga* isolates; however, it did prevent the colonization of *S. mutans* (data not shown). Finally, plaques formed on wires by both *S. mutans* and *Cytophaga* strains were treated with an endodextranase (data not shown). At the end of the 24-h incubation period, the *S. mutans* plaque sample had been reduced to less than half of its original size, whereas the *Cytophaga* plaque sample remained unchanged. Therefore, it appears that dextran is not a significant component of the *Cytophaga* plaque deposit.

Examination of the root surface after 3 weeks of colonization by these microorganisms revealed that the layer of cementum and underlying dentin had been degraded and demineralized. Gentle probing with a scalpel dislodged large pulpy sections of the root. The consistency of the material was very similar to that found in carious lesions produced by streptococci.

## DISCUSSION

The slender, filamentous, gram-negative rods isolated from subgingival plaque of patients with advanced periodontal disease are morphologically similar to microorganisms photographed in plaque deposits taken from the apical areas of periodontal lesions (9). Tentatively identified as members of the *Cytophaga* in our laboratory, these isolates exhibited a predilection for colonizing the root surface of intact teeth or the dentin surface of sectioned teeth; the organisms do not adhere well to the smooth enamel surfaces. Their apparent specificity for the cementum surface of the root and dentin surfaces of sectioned teeth suggests that these microorganisms may be binding to one or more structural proteins present in the ground substance of those sections of the tooth. Studies are currently underway to determine the specific binding sites of these microorganisms.

The ability to attach to the cementum surface of the root and produce acid from a limited number of sugars (unpublished data) endows these microorganisms with an obvious pathogenic potential. If the in vitro experiments with intact teeth even remotely simulate events occurring in vivo, these organisms may contribute significantly to the progression of periodontal disease. Their contribution to the diseased state can be made in one of several ways. By simply binding to the root surface, these gram-negative microorganisms may provide a localized concentrated source of lipopolysaccharide on or near the alveolar bone-root juncture which could cause bone resorption by eliciting a host-mediated immune response (13). In this connection, it should be noted that the cementum surface of teeth extracted from patients with periodontal disease contains tightly bound endotoxin-like material (1). However, the bacterium itself is capable of demineralizing the dentin layer of the root and could conceivably demineralize bone. Destruction of the former would require the presence of a fermentable carbohydrate and may manifest itself as a carious root lesion. A concerted effort to define the role of this organism in periodontal disease is presently underway.

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