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Immune Labeling of Certain Strains of *Actinomyces naeslundii* and *Actinomyces viscosus* by Fluorescence and Electron Microscopy

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A total of 12 well-characterized strains of *Actinomyces viscosus* and *A. naeslundii* grown on Trypticase soy agar plates supplemented with sheep erythrocytes were examined by light microscopy and transmission electron microscopy after treatment with appropriately labeled antisera to homologous and heterologous strains. Cells incubated with homologous rabbit antisera followed by fluorescein-isothiocyanate (FITC)-conjugated goat anti-rabbit immunoglobulin G (IgG) exhibited a completely smooth fluorescent outline in the case of *A. naeslundii* and an interrupted, irregular fluorescent outline in the case of human strains of *A. viscosus*. The different labeling patterns appeared to be related to the presence at the ultrastructural level of long, unevenly distributed strands of "fuzz" on the surface of human *A. viscosus* cells, whereas *A. naeslundii* cells had a narrower layer of fuzz, of more even thickness. The immunocoating reaction revealed homologous antibody binding to the irregular strands of fuzz on the surface of human *A. viscosus* cells, whereas homologous antisera to *A. naeslundii* coated *A. naeslundii* cells with a moderately electron-dense coating of antibody of even thickness. Human strains of *A. viscosus* incubated with heterologous antiserum to *A. naeslundii* followed by FITC-labeled goat anti-rabbit IgG exhibited a segmented fluorescent outline, which differed from that produced with homologous antisera. *A. naeslundii* incubated with heterologous rabbit antisera to human *A. viscosus* strains and FITC-labeled anti-rabbit IgG exhibited a completely smooth fluorescent outline similar to that produced with homologous antiserum. *A. viscosus* strains of hamster origin differed from *A. viscosus* strains of human origin by the absence of a surface fuzz and the comparatively smooth, even fluorescence produced by incubating these cells with homologous rabbit antiserum followed by FITC-labeled goat anti-rabbit IgG. Antiserum to a hamster strain did not cross-react with *A. naeslundii* or human strains of *A. viscosus*. Under the growth conditions of this experiment, ultrastructural features and labeling patterns with the indirect fluorescent technique may be useful in differentiating these serotypes from one another.

Actinomyces naeslundii and *A. viscosus* are common inhabitants of the oral cavity which have been implicated as potential pathogens for both periodontal disease (12, 14, 20) and root caries (18) in humans and experimental animals (3, 5, 9, 17). Biochemically, these species closely resemble each other, the main distinguishing feature being that *A. viscosus* is catalase positive and *A. naeslundii* is catalase negative. On the basis of serological cross-reactions, Gerencser and Slack (7) subdivided *A. viscosus* into two serotypes and *A. naeslundii* into 3 or 4 serotypes.

One-way and two-way cross-reactions of various intensities which have been reported among the various serotypes may make it difficult to identify these with certainty, although cross-reactions are said to be usually strain variable and low titer (7).

In the course of attempts at labeling various strains of *A. viscosus* and *A. naeslundii* in our laboratory by the indirect immunofluorescence technique, differences were noted in the labeling patterns of the strains tested with homologous as well as heterologous antisera. Additional experiments were carried out to determine if ultrastructural differences among the strains could be associated with differences in the patterns of antibody binding at the cell surface.

In this report the ultrastructural features of

A. naeslundii and *A. viscosus* will be reviewed with emphasis on certain features which may be helpful in distinguishing among certain serotypes. The immunocoating reaction (4, 11, 19), which allows the ultrastructural localization of surface antigens by specific antibody without the use of electron-dense markers, such as ferritin or horseradish peroxidase, was used to study the pattern of antibody binding at the cell surface.

MATERIALS AND METHODS

Strains and cultural conditions. A total of 12 well-characterized strains were selected. These included four strains of *A. naeslundii* serotype 1 (ATCC 12104, WVU 509, WVU 398A, and WVU 45), two animal strains of *A. viscosus* serotype 1 (T6 and WVU 440), and six human isolates of *A. viscosus* serotype 2 (ATCC 19246, RC45, T14V, WVU 505, WVU 472, and WVU 371). The identity of each strain was confirmed by appropriate biochemical tests (16) carried out by conventional methods and the Minitek system (BBL Microbiology Systems, Cockeysville, Md.).

All strains were grown anaerobically on Trypticase-soy agar plates supplemented with 5% sheep erythrocytes (BBL) at 35°C for 48 h. The cells were harvested by gently scraping colonies from the agar surface and suspending them in 0.05 M phosphate-buffered saline. The cells were collected by centrifugation and processed for ultrastructural study of intact cells, ultrastructural study of cells after immunocoating, and immunofluorescent studies.

Preparation of antisera. Antisera were produced in rabbits against *A. viscosus* strains RC45, ATCC 19246, and T6 and *A. naeslundii* strain ATCC 12104. Rabbits were injected intravenously every 2 days, three times weekly, for 4 weeks with 0.2 ml of a suspension of live cells, prepared at a concentration of 600 g of N/ml. At the end of 4 weeks the antisera were collected by intracardiac puncture.

Indirect fluorescent antibody technique. Smears were prepared on glass slides from cells suspended in phosphate-buffered saline. The cells were heat fixed and incubated for 30 min at 25°C with serial dilutions (1:10 to 1:640) of the four rabbit antisera listed above. Excess antiserum was removed by repeated washings in 0.05 M phosphate-buffered saline. The smears were then incubated with fluorescein-isothiocyanate-conjugated goat anti-rabbit immunoglobulin G (IgG) (Miles Laboratories, Kankakee, Ill.) for another 30 min and washed overnight at 4°C to remove any unbound conjugated antibody. Controls included smears treated with preimmune (normal) rabbit sera instead of antisera, followed by fluorescein-isothiocyanate-conjugated goat anti-rabbit IgG, and smears were incubated with fluorescein-isothiocyanate-conjugated goat anti-rabbit IgG only. The slides were examined with a Zeiss microscope equipped with a halogen illuminator (12 V, 100 W), fluorescein-isothiocyanate exciter filter, no. 50 and no. 53 barrier filters, and a BG 38 filter.

Fluorescence was considered to be positive if the cell wall had a visually detectable fluorescent outline. Depending on its intensity, the positive reaction was considered to be either weak, i.e., detectable but not bright, or strong, i.e., characterized by a bright peripheral outline. For the purpose of this study, the titer of an antiserum was taken as the reciprocal of the highest dilution to produce a strong, positive fluorescence.

Immunocoating. Immunocoating was done according to the procedure of Lai et al. (11). After centrifugation, the cells of each strain were divided into aliquots of packed cells, approximately 0.25 ml in volume. Each of four aliquots was resuspended in a volume of 0.5 ml of undiluted rabbit antiserum, using each of the four antisera. The suspended cells were incubated at 25°C for 30 min and washed in phosphate-buffered saline by repeated centrifugation to remove any excess antibody. The cells were then incubated with goat anti-rabbit IgG for another 30 min (double immunocoating) to enhance the localization of antibody at the cell surface. After a double wash in phosphate-buffered saline, the cells were pelleted and immediately fixed and processed for electron microscopy. Controls were prepared by incubating each strain with normal rabbit serum instead of antiserum, followed by goat anti-rabbit IgG, and by incubating each strain with goat anti-rabbit IgG only.

Electron microscopy. Pellets of freshly harvested cells or immunocoated cells were immediately prefixed in a mixture of 5% glutaraldehyde and 4% paraformaldehyde in 0.1 M sodium cacodylate buffer at pH 7.2 (10) and subdivided into smaller blocks measuring no more than 1 mm in any dimension. After several rinses in 0.2 M s-collidine buffer (Eastman, Rochester, N.Y.) with 4% sucrose added, the specimens were postfixed in 2% s-collidine-buffered osmic acid (pH 7.2, 350 milliosmoles) for 2 h at 4°C, dehydrated in graded ethanol solutions, and embedded in Epon (13). Sections cut at 0.1 μm were collected on uncoated grids, stained with uranyl acetate and lead citrate, and examined and photographed in a Philips EM-300 electron microscope.

RESULTS

Indirect fluorescent antibody labeling. Table 1 summarizes the results of the serological cross-reactions between the bacterial strains and the four antisera. The various strains included under each serotype generally behaved in essentially the same manner. All strains stained strongly with antisera homologous to their serotypes. (Fig. 1A through C).

A. naeslundii serotype 1 strains reacted only moderately with the antisera to *A. viscosus* serotype 2 strains and did not react with antiserum to the *A. viscosus* serotype 1 strain. Whenever *A. naeslundii* cells were labeled, the label was distributed evenly, as an uninterrupted bright line surrounding the cell.

A. viscosus serotype 2 cells reacted strongly with the heterologous antiserum to *A. naeslundii* ATCC strain 12104, as well as with antisera to *A. viscosus* serotype 2 strains RC45 and ATCC 19246. With the latter two antisera, the label was distributed either as an even, uninter-

TABLE 1. *Serological reactions of A. naeslundii and A. viscosus strains based on the indirect fluorescent antibody technique*

Strain tested	Cross-reaction[a] with antiserum to:			
	A. naeslundii ATCC 12104	*A. viscosus* RC45	*A. viscosus* ATCC 19246	*A. viscosus* T6
A. naeslundii serotype 1				
ATCC 12104	+++ A	++ A	++ A	–
WVU 509	+++ A	++ A	++ A	–
WVU 398A	+++ A	++ A	++ A	–
WVU 45	+++ A	++ A	++ A	–
A. viscosus serotype 2				
RC45	+++ C	+++ B	+++ B	–
T14V	+++ C	+++ B	+++ B	–
WVU 472	+++ C	+++ B	+++ B	–
WVU 505	+++ C	+++ B	+++ B	–
WVU 371	+++ C	+++ A	+++ A	–
ATCC 19246	+++ C	+++ A	+++ A	–
A. viscosus serotype 1				
T6	+ A	+ A	+ A	+++ A
WVU 440	+ A	+ A	+ A	+++ A

[a] –, No labeling; +, weakly positive at low dilutions (1:10 or 1:20); ++, strongly positive at moderate dilutions (1:40 or 1:80); +++, strongly positive at high dilutions (1:160 or 1:320); A, completely smooth fluorescent outline; B, interrupted, irregular fluorescent outline with peripheral projections; C, segmented fluorescent outline, with unlabeled poles and septal regions.

rupted layer along the cell periphery or as a bright, rough line of irregular width, sometimes with minute interruptions (Fig. 1B). The latter was the more common labeling pattern at higher dilutions (1:160 to 1:640). Strains ATCC 19246 and WVU 371 only exhibited an even, uninterrupted pattern of labeling. Like the cells of *A. naeslundii* serotype 1 strains, *A. viscosus* serotype 2 cells were not labeled with *A. viscosus* serotype 1 antiserum to T6. With the antiserum to *A. naeslundii*, although the reaction was strong, the labeling pattern was quite distinctive inasmuch as it was not evenly distributed around the cell. The brightest labeling was located on that portion of the cell wall interposed between cell ends, including ends recently formed by cell division. This gave the labeling a segmented appearance distinct from that produced by homologous antisera (Fig. 1D).

Both *A. viscosus* serotype 1 strains reacted strongly with the homologous antiserum to T6. The label appeared as a bright, smooth, uninterrupted outline around each cell. With antisera to *A. naesulundii* and *A. viscosus* serotype 2 strains, the serotype 1 strains reacted weakly, but the label was evenly distributed along the cell periphery.

No immunofluorescence was detected in any of the control preparations exposed to normal (preimmune) rabbit serum followed by goat anti-rabbit IgG or to goat anti-rabbit IgG alone.

Ultrastructural features. The general appearances of representative cells from the three serotypes included in this study are shown in Fig. 2. An obvious difference among these serotypes at this magnification is the presence of a well-developed "fuzz" on the surface of *A. viscosus* serotype 2 cells (Fig. 2B), a much narrower band of fuzz on the surface of *A. naeslundii* cells (Fig. 2A), and the absence of a surface fuzz on the *A. viscosus* serotype 1 cells (Fig. 2C). The thickness of the fuzz of *A. viscosus* serotype 2 cells generally measured more than twice the width of the cell wall. This was not true for strains ATCC 19246 and WVU 371, which had an ill-defined layer of fuzz. The individual strands varied greatly in length, frequently branching and connecting with strands from neighboring cells. This was particularly evident in cells immunocoated with homologous antiserum (see Fig. 5A). By contrast, *A. naeslundii* cells had a narrow fuzz covering, less than twice the width of the cell wall, consisting of strands of more or less even length (Fig. 2A).

The basic structure of the cell periphery was similar to that of typical gram-positive cells (Fig. 3). However, certain differences were noted among the three serotypes grown under these particular experimental conditions. The typical cell periphery consisted chiefly of the cell wall per se (CW), which was composed of two distinct layers, an external, moderately electron-dense structure (CW_E) which formed the main component of the CW, and an electron-dense internal layer (CW_I) generally in contact with the plasma membrane (Fig. 3C through F). The plasma membrane consisted of an internal electron-dense layer and an external electron-dense layer separated by an electron-lucent layer. In general, the CW_I was in direct contact with the external plasma membrane so that these could not always be resolved as individual layers (Fig. 3C through F). In some cells of the *A. naesulundii* and *A. viscosus* serotype 1 strains a periplasmic space separated the CW from the plasma membrane so that the CW_I was distinguishable from the external plasma membrane (PM_E in Fig. 3G and H).

A. viscosus serotype 2 strains (Fig. 3A and B) differed from the other serotypes (Fig. 3C through H) by the lack of a well-defined CW_I. In the cells of these strains the CW_I was barely distinguishable from the external CW because the electron density of these layers was almost the same and in the range observed for the external CWs of the other serotypes. In a num-

ber of cells the CW_I appeared to be missing altogether. When this was the case, both electron-dense layers of the plasma membrane could be clearly resolved even in the absence of a separation between the cell membrane and the CW.

Immunocoating. When *A. naeslundii* serotype 1 cells were exposed to homologous rabbit antiserum followed by goat anti-rabbit IgG, the cells became coated with a distinct layer of intermediate electron density approximately 30 to 75 nm thick (Fig. 4A) which completely sur-

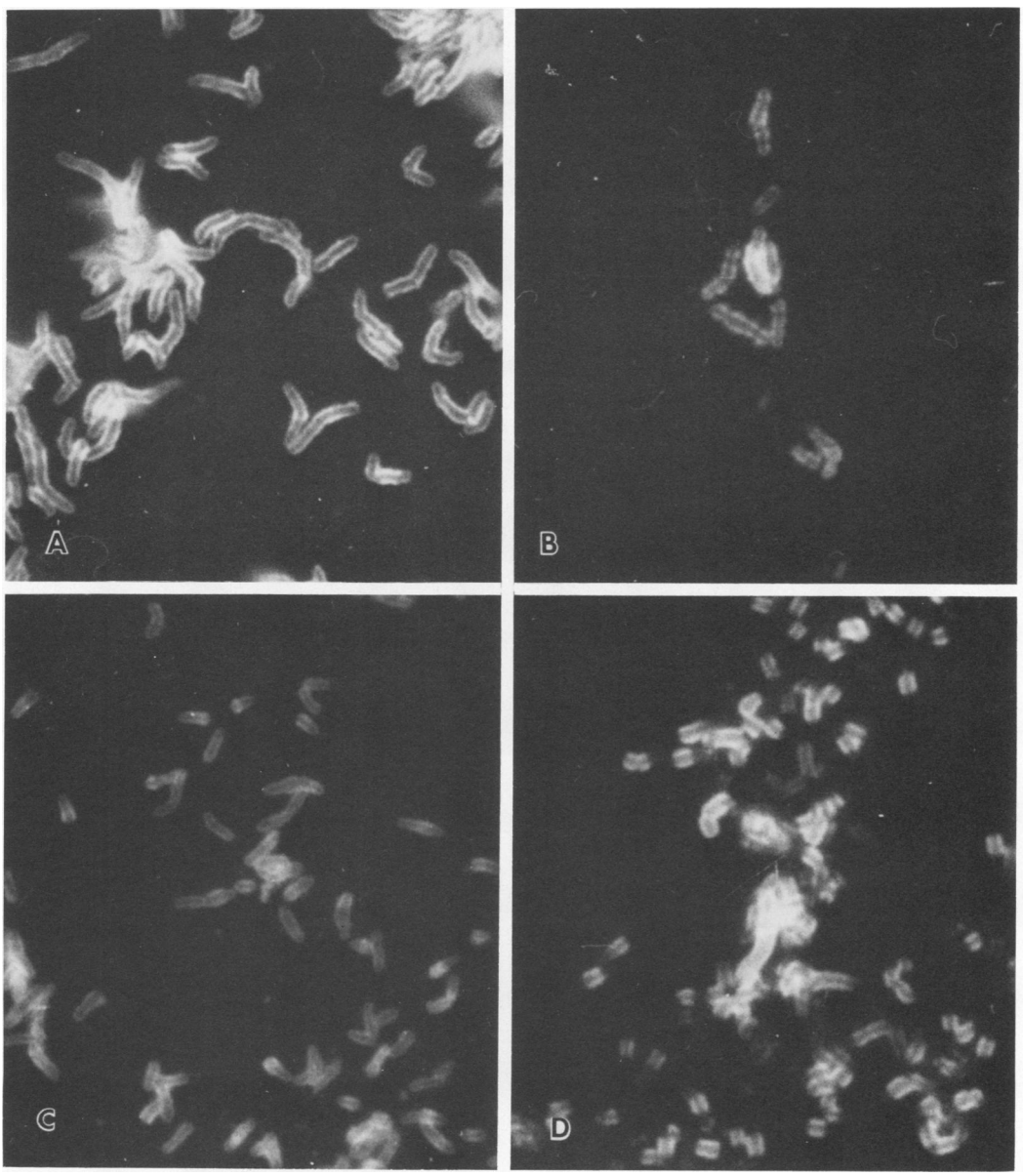

FIG. 1. *Photomicrographs of indirect fluorescent antibody preparations. (A) A. naeslundii strain ATCC 12104 labeled with homologous antiserum; note that the label produces a bright, smooth, uninterrupted outline around each cell. (B) A. viscosus strain RC45 labeled with homologous antiserum; note the rough, irregular outline of the label with minute interruptions. (C) A. viscosus strain T6 labeled with homologous antiserum; the labeling pattern is similar to that in (A). (D) A. viscosus strain RC45 labeled with heterologous antiserum to A. naeslundii strain ATCC 12104; note segmented distribution of the label. ×2,800.*

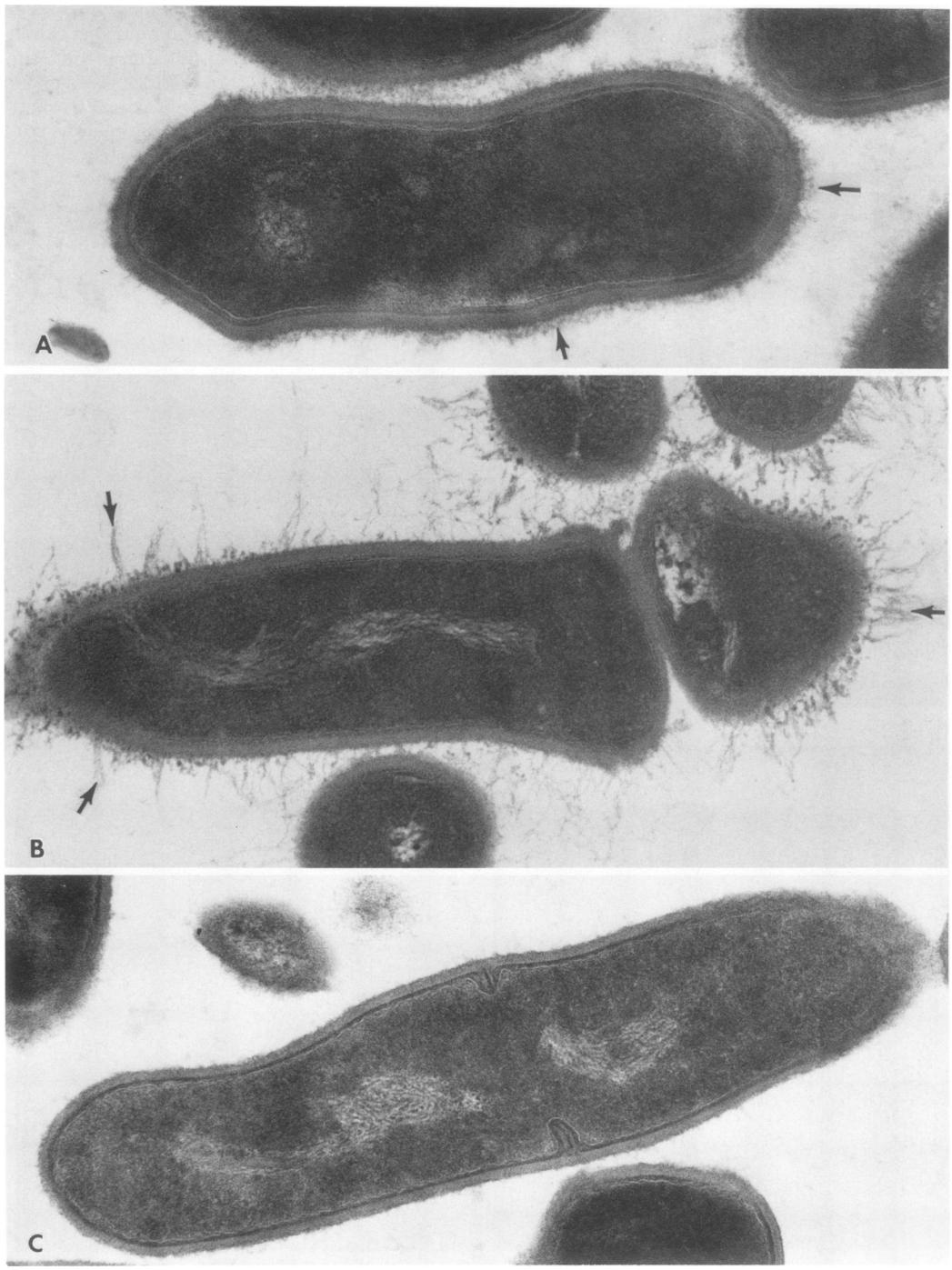

FIG. 2. *Electron micrographs of typical cells. (A) A. naeslundii serotype 1, strain ATCC 12104; note the presence of short strands of surface fuzz of more or less even length (arrows). (B) A. viscosus of human origin (serotype 2), strain RC45; note the distinct relatively long strands of surface fuzz surrounding the cell (arrows). (C) A. viscosus of hamster origin (serotype 1), strain T6; note the absence of a surface fuzz. ×65,700.*

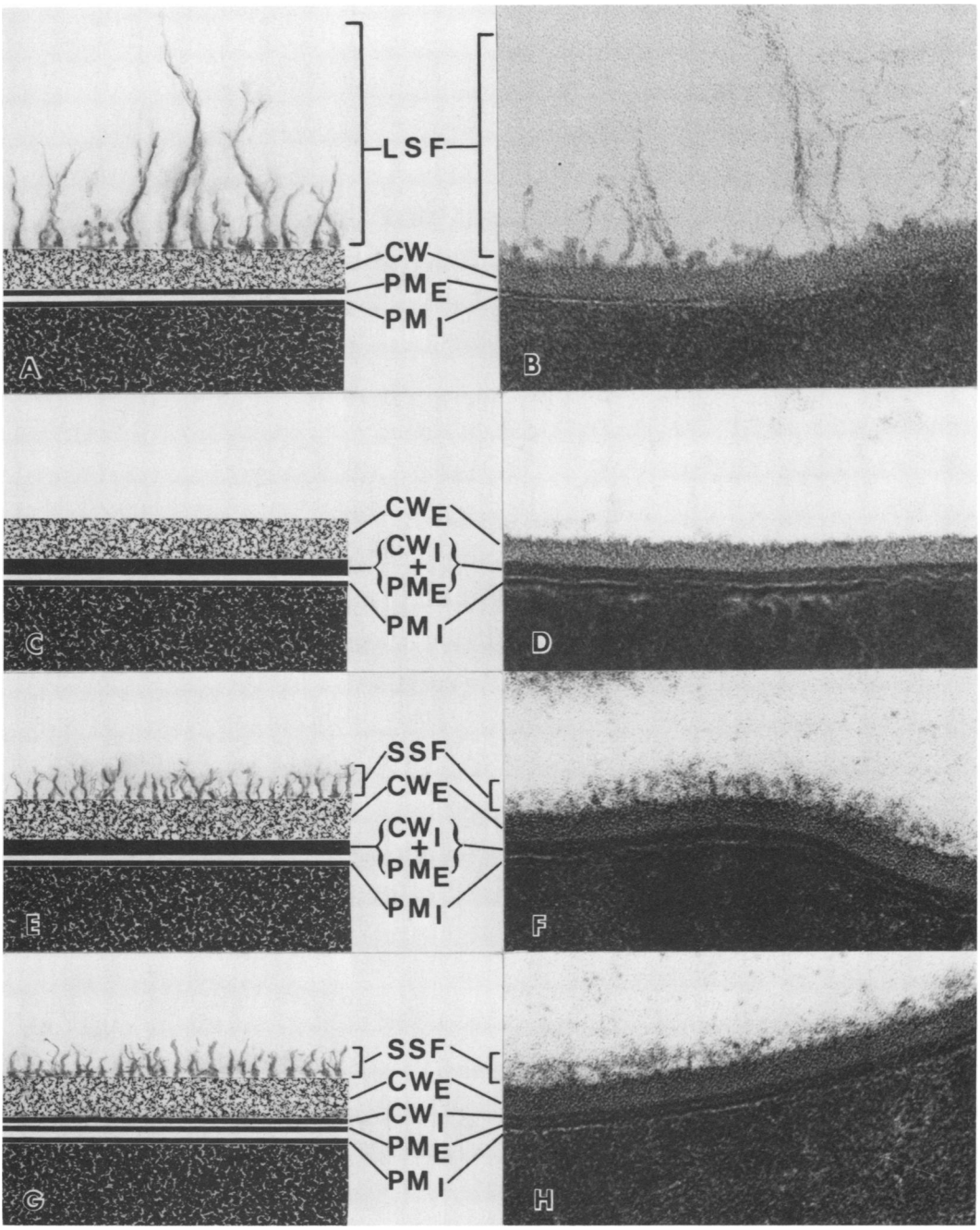

FIG. 3. *Highly magnified electron micrographs and accompanying diagrams of the basic structures of the cell wall (CW) and cell periphery. (A and B) A. viscosus of human origin (serotype 2). (C and D) A. viscosus of hamster origin (serotype 1). (E through H) A. naeslundii (serotype 1). The CW per se is composed of a moderately dense external layer (CW_E) and a dense internal layer (CW_I) which can be distinguished in (G) and (H). The dense CW_I may be in direct contact with the dense external layer of the plasma membrane (PM_E), thereby giving rise to a combined, thicker electron-dense layer ($CW_I + PM_E$) as shown in (C) through (F). Note the absence of the CW_I in human strains of A. viscosus (A and B). Abbreviations: PM_I, internal layer of the plasma membrane; LSF, long surface fuzz; SSF, short surface fuzz. ×196,800.*

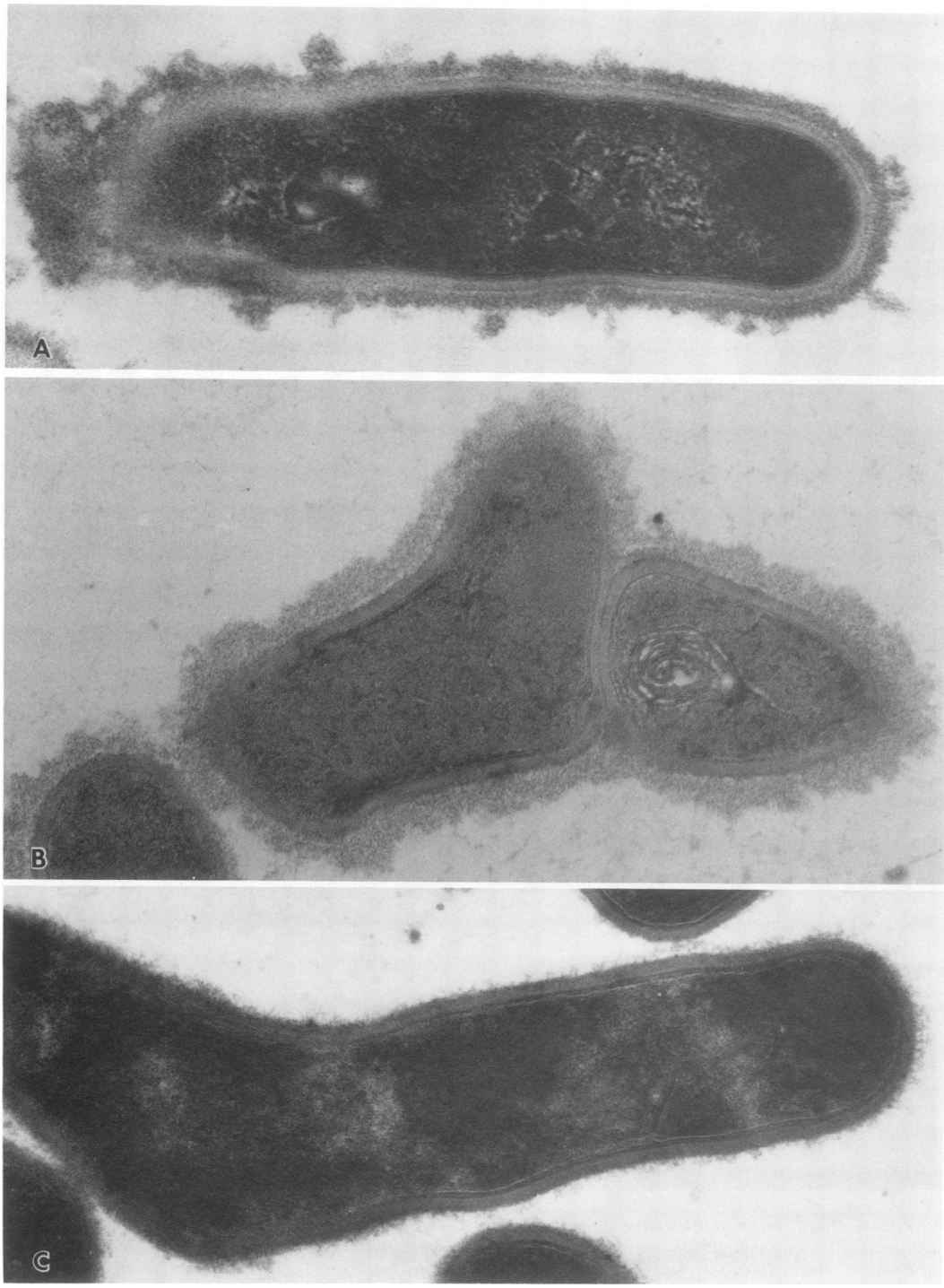

FIG. 4. *Immunocoating of A. naeslundii strain ATCC 12104 with homologous antiserum (A), and heterologous antisera to the human strain A. viscosus RC45 (B) and to the hamster strain A. viscosus T6 (C). Incubation of the cells with the rabbit antisera was followed by an incubation with goat anti-rabbit IgG. Note the reaction products in (A) and (B) and the lack of immunocoating in (C). ×68,000.*

rounded the cell wall. A similar type of coating developed when these cells were exposed to the antisera to *A. viscosus* serotype 2 strains followed by goat anti-rabbit IgG (Fig. 4B). When a coating was formed, it appeared to be localized to the narrow zone of fuzz surrounding the cell wall. No immunocoating was detected after *A. naeslundii* cells were exposed to antiserum to *A. viscosus* serotype 1 followed by goat anti-rabbit IgG (Fig. 4C).

When *A. viscosus* serotype 2 cells were exposed to either one of the *A. viscosus* serotype 2 antisera followed by goat anti-rabbit IgG, the distribution of the coating was distinct from that seen for the other serotypes. In most of the strains of this serotype (ATCC 19246 and its derivative WVU 371 excepted) the strands of fuzz were clearly outlined by the coating which covered the strands and also surrounded the CW (Fig. 5A). In some of the cells, the coating surrounding the CW was separated from the CW per se by an electron-lucent zone approximately as wide as the coating, which contained fine filaments radiating from the CW surface to the coating as well as isolated strands of coated fuzz. The presence of this electron-lucent zone appeared limited to portions of the cell surface bearing electron-dense granules, approximately 10 to 30 nm in diameter (Fig. 5B). When the ATCC 19246 and WVU 371 strains were exposed to either of the two antisera against *A. viscosus* serotype 2 followed by goat anti-rabbit IgG, a relatively uniform coating was formed on the cell surface, with an appearance similar to that reported above for *A. naeslundii* cells reacted with antiserum to *A. naeslundii* (Fig. 4A).

In the presence of antiserum to *A. naeslundii* followed by goat anti-rabbit IgG, the *A. viscosus* serotype 2 cells were coated in a patchy manner along the strands of fuzz radiating from the surface. The immunocoating material was deposited in more or less globular clumps, approximately 30 to 40 nm wide, with frequent gaps between adjacent clumps (Fig. 6A). No immunocoating was detectable when *A. viscosus* serotype 2 cells were reacted with antiserum to the *A. viscosus* serotype 1 strain T6 followed by goat anti-rabbit IgG (Fig. 6B).

When *A. viscosus* serotype 1 strains were exposed to homologous antiserum followed by goat anti-rabbit IgG, a distinct, continuous but uneven coat was formed on the cell wall surface. The thickness of the coating ranged from 30 to 130 nm, with isolated areas measuring up to 250 nm (Fig. 7A). In the presence of heterologous antiserum against either an *A. naeslundii* or an *A. viscosus* serotype 2 strain, only patches of electron-dense material separated by wide, non-labeled gaps appeared along the cell surface (Fig. 7B and C).

No immunocoating was detected in any of the control strains exposed to normal (preimmune) rabbit serum followed by goat anti-rabbit IgG or to goat anti-rabbit IgG only.

DISCUSSION

The results obtained in this study with indirect fluorescent antibody labeling duplicate in part those of other investigators who have attempted to label various *Actinomyces* species with fluorescent antibodies. Thus, Bellack and Jordan (1) reported that high-titer antisera to rodent strains of *A. viscosus*, including T6, labeled homologous cells but did not generally label *A. viscosus* cells of human origin. No antisera to human strains were tested on the animal strains. Gerencser and Slack (6) pointed out that animal strains of *A. viscosus* (serotype 1) were serologically distinct from human strains (serotype 2). Furthermore, A. L. Coykendall, T. W. Lee, and A. T. Brown (Int. Assoc. Dent. Res. Gen. Meet. 52nd, Abstr. no. 74, 1974) demonstrated sufficiently great discrepancies in percent guanine plus cytosine among animal and human strains to warrant placing them in different species. Holmberg and Forsum (8) noted that "dilution of conjugates eliminated most cross-reactions at low titers and rendered the conjugates species specific at dilutions of 1:32 to 1:64." To avoid nonspecific reactions with *Staphylococcus aureus* protein A, they recommended the use of $F(ab^1)_2$ fragments rather than whole IgG. It should be noted that *S. aureus* is an infrequent member of the periodontal flora. Therefore, nonspecific cross-reactions with protein A should not constitute a serious problem when localization of selected microorganisms in dental plaque is attempted.

Our observation that *A. naeslundii* serotype 1 and *A. viscosus* serotype 2 strains failed to react with antiserum to *A. viscosus* serotype 1 but that the reverse was not true, at least at low titers, agrees with the statement by Gerencser and Slack (7) that low-titer, one-way cross-reactions can be expected between *A. naeslundii* or *A. viscosus* serotype 2 and *A. viscosus* serotype 1. Whereas a number of reports dealing with immunofluorescent labeling of *Actinomyces* cells have indicated that various intensities of labeling occur, variation in the distribution of the label on the cell surface has not been given much attention. It would appear from our observations that, with the exception of ATCC 19246 and its derivative WVU 371, the distribution of the fluorescent label on the cell surface may also be useful in distinguishing among the

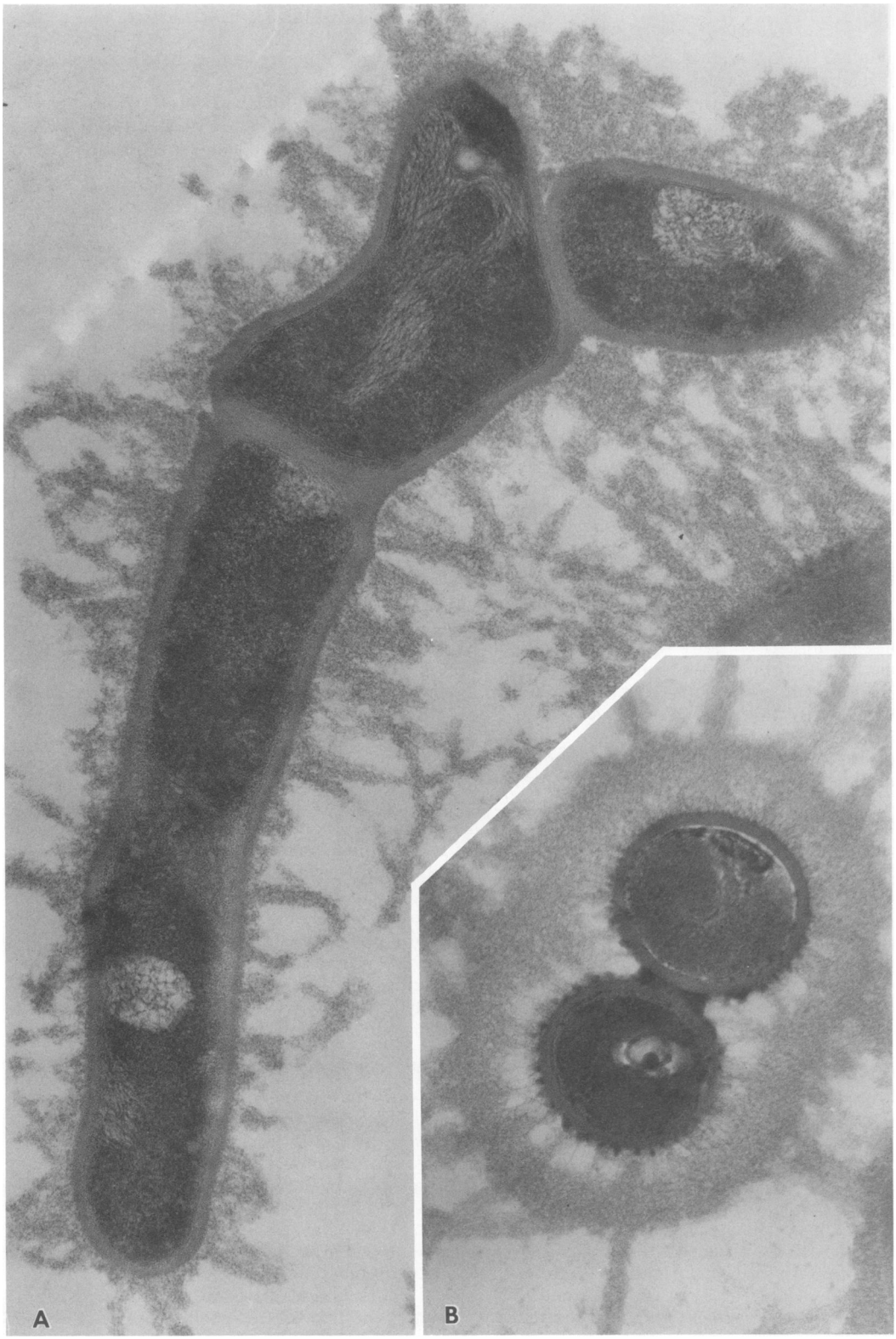

FIG. 5. *Immunocoating of A. viscosus strain RC45 with homologous antiserum followed by goat anti-rabbit IgG. (A) Localization of the label on the strands of fuzz and the CW periphery. (B) Electron-lucent zone opposite the granular portion of the cell surface.* ×68,000.

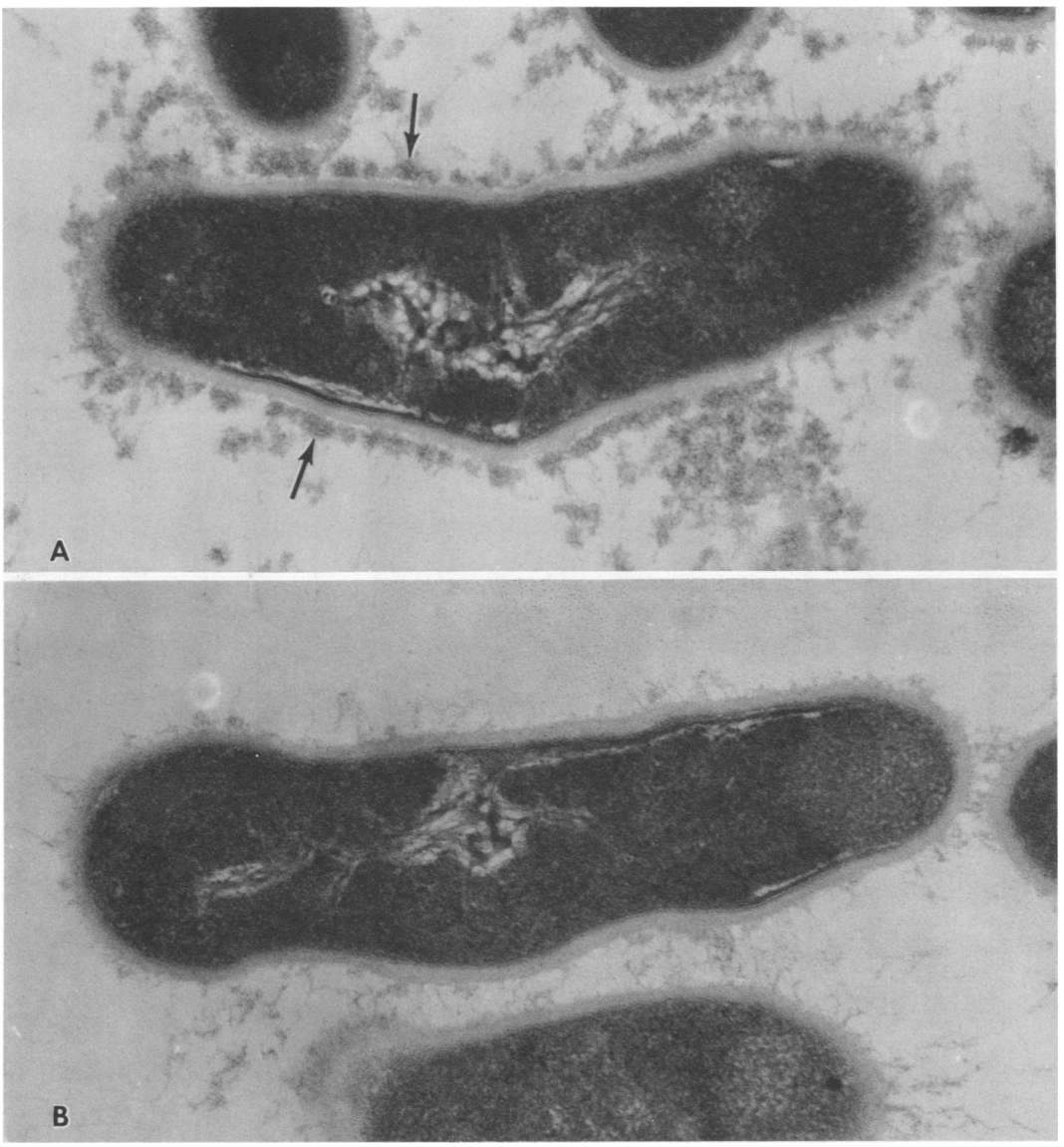

FIG. 6. *Immunocoating of A. viscosus strain T14 incubated with heterologous antiserum to A. naeslundii strain ATCC 12104 (A) or heterologous antiserum to A. viscosus strain T6 (B). Note the patchy immunocoating (arrow) characteristic of a weak cross-reaction with antiserum to A. naeslundii (A) and the lack of immunocoating typically observed with T6 antiserum (B).* ×*68,000.*

serotypes included in this report (Table 1).

The explanation for the differences in the labeling patterns with the indirect fluorescent antibody technique may be derived in part from the observations at the ultrastructural level of unlabeled and labeled cells. Thus, *A. naeslundii* cells and *A. viscosus* serotype 1 cells all exhibit relatively smooth surfaces. Although a fuzz layer is present on the surface of *A. naeslundii* cells, it is of even thickness with a relatively smooth outer surface. Both of these cell types exposed to homologous antisera, followed by goat anti-rabbit IgG, exhibit a relatively even, uninterrupted, moderately electron-dense layer of immune complexes, which represents the ultrastructural equivalent of the fluorescent band.

In contrast, *A. viscosus* serotype 2 cells are characterized by a fuzz composed of long strands radiating from the cell surface, many of which are of different lengths and are distributed along

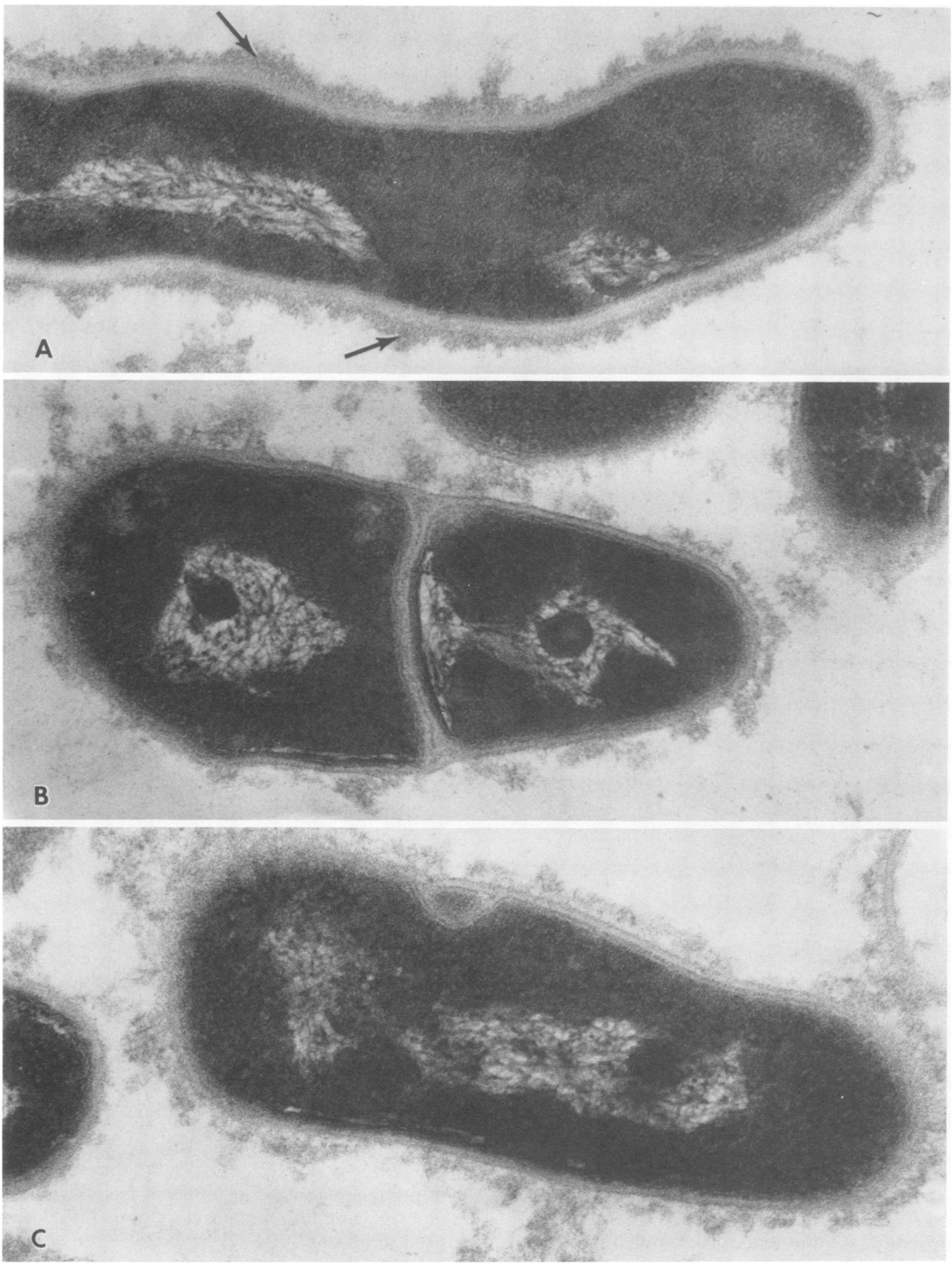

FIG. 7. *Immunocoating of A. viscosus strain T6 exposed to homologous antiserum (A), heterologous antiserum to A. viscosus strain RC45 (B), and heterologous antiserum to A. naeslundii strain 12104 (C) followed by goat anti-rabbit IgG. Note the continuous immunocoating layer which surrounds the cell wall in (A) (arrows) and the weak, patchy immunocoating layer (arrows) along the cell periphery in (B) and (C).* ×*68,000.*

the cell surface in arrays of different densities. It is apparent from cells labeled with homologous antiserum that the label has a strong affinity for the strands of fuzz, an observation which is corroborated by Cisar et al. (2). These investigators demonstrated by electron microscopy the selective labeling of fuzz strands of *A. viscosus* T14 "virulent" and "avirulent" cells with antibody to a virulence-associated antigen. The distribution of the label was similar to that shown in this study when *A. viscosus* serotype 2 cells, including strain T14V, were exposed to whole cell antiserum against strain RC45 or ATCC 19246. These ultrastructural observations explain the uneven periphery noted by immunofluorescence for most *A. viscosus* serotype 2 cells labeled with homologous antiserum. What is not readily explainable from the ultrastructural data is the cause of the minute interruptions observed in the fluorescent layer after *A. viscosus* serotype 2 cells have been reacted with high titers of homologous antisera or the reason for the segmented distribution of the fluorescent label after treatment of these cells with *A. naeslundii* antiserum. None of the labeled *A. viscosus* serotype 2 cells examined with the electron microscope demonstrated clearly defined, nonlabeled regions on the cell surface which could account for the interrupted outline observed with the indirect fluorescent antibody technique.

In contrast to the other serotype 2 strains, ATCC 19246 and WVU 372 labeled with homologous antisera exhibited a continuous, uninterrupted outline by immunofluorescence. From the ultrastructure of labeled and unlabeled cells it was apparent that these cells lacked the well-developed fuzz typical of the majority of serotype 2 cells. Consequently, the labeling pattern for these two strains tended to resemble that reported for *A. naeslundii* labeled with homologous serum rather than that for *A. viscosus* serotype 2 cells labeled with homologous serum. It should be noted, however, that ATCC 19246 and WVU 371 behaved like other *A. viscosus* serotype 2 strains in terms of their labeling patterns when reacted with heterologous antisera.

More recently, Powell et al. (15) contributed some new information on the ultrastructural features of the virulent (T14V) and avirulent (T14AV) varieties of T14. Whereas both types of T14 had fuzz-covered surfaces, T14AV strains grown in Trypticase soy broth supplemented with 1% glucose and 0.1% yeast extract possessed a capsule which partially masked the bases of the fibrils. In addition, the virulent strain appeared more fibrillar than the avirulent strain under certain cultural conditions. However, both strains appeared equally capable of adsorbing "virulence-associated antigens," a finding which suggests that the virulent and avirulent varieties of *A. viscosus* T14 are similar with respect to their antigenic properties, at least qualitatively if not quantitatively.

Eisenberg and Montgomery (4) reported uniform labeling of normal *Streptococcus sanguis* cells with homologous antiserum by means of the immunocoating reaction (11) as well as by immunofluorescence. However, the distribution of the label differed when the *S. sanguis* cells were rendered pleomorphic by growing them in the presence of oxygen. When such cells were labeled with homologous antisera, cell surfaces adjacent to septal regions were devoid of label both at the ultrastructural level and by immunofluorescence. From the above as well as other reports, it is evident that cultural conditions can influence the morphology of certain surface features. Thus, it is necessary to emphasize that the microscopic observations reported here are valid only for cells grown under the conditions described.

Among the *A. viscosus* serotype 2 strains, ATCC 19246 or its homolog, WVU 371, seemed to behave differently from the other strains when it was reacted with antiserum to ATCC 19246 or RC45. Instead of exhibiting the characteristic rough outline with multiple interruptions seen in immunofluorescent preparations of other serotype 2 strains, this strain reacted with the formation of a smooth, uninterrupted layer of fluorescence on the cell surface. The ultrastructure of this strain revealed a lack of long-stranded fuzz on the cell surface. After immunocoating with homologous antisera, a smooth, relatively even coating was produced similar to that seen with *A. naeslundii* cells immunocoated with homologous antiserum. It is of interest to note that ATCC 19246 and its homolog, WVU 371, were the only strains of this serotype not isolated from calculus or dental plaque. The original strain was derived from a case of cervicofacial actinomycosis. Furthermore, it is likely that since its isolation it has undergone changes in its serological characteristics which suggest that it may no longer be representative of *A. viscosus* serotype 2 (M. A. Gerencser, personal communication).

From our results, it is evident that under the cultural conditions reported here, the presence, size, and distribution of the fuzz layer may be important structural criteria in differentiating *A. naeslundii* serotype 1 and *A. viscosus* serotypes 1 and 2 from one another. The absence or lack of a well-defined CW_I may be an additional structural feature worth considering in differentiating *A. viscosus* serotype 2 from *A. viscosus*

serotype 1 and *A. naeslundii* strains. Finally, the pattern of distribution at the cell surface of immunofluorescent labels as well as their intensities at different titers may be helpful in differentiating among these serotypes.

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