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Presence of Pili in Species of Human and Animal Parasites and Pathogens of the Genus *Corynebacterium*

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The presence of pili in human and animal parasites and pathogens of the genus *Corynebacterium* was examined. *C. kutscheri*, *C. diphtheriae*, and *C. pseudodiphtheriticum* possessed a fairly large number of pili, ranging from dozens to more than a hundred, in 91 to 100% of the bacterial cells. *C. equi*, *C. hoagii*, *C. xerosis*, *C. pyogenes*, and *C. murisepticum* had only a small number of pili in 10 to 37% of the bacterial cells. In *C. bovis*, *C. striatum*, and *C. pseudotuberculosis*, pili were detected in only 0.5 to 3% of the bacterial cells. The pili were similar to each other and to those of *C. renale;* they were not rigid and had a tendency to form bundles. The length of pili usually ranged from 0.2 to 3 μm, and their diameter was within a 2- to 6-nm range.

Bacterial pili were first found in gram-negative bacteria (1, 3, 8). At present, pili seem to be restricted mainly to gram-negative bacteria, because similar filamentous appendages have been observed only on the gram-positive *Corynebacterium renale* (13, 15, 16). Morphological, chemical, and immunological properties (11, 12, 15, 16) and adhesive characteristics (9, 10) of the pili of *C. renale* have been reported. As yet, however, it has not been determined whether or not other species of *Corynebacterium* possess pili. The present study deals with the subject using corynebacteria of human and animal parasites and pathogens.

The strains of *Corynebacterium* used were as follows: *C. diphtheriae* (ATCC 19409), *C. pseudotuberculosis* (ATCC 19410), *C. xerosis* (ATCC 373), *C. kutscheri* (ATCC 15677), *C. pseudodiphtheriticum* (ATCC 10700), *C. equi* (ATCC 6939), *C. bovis* (ATCC 7715), *C. pyogenes* (ATCC 19411), *C. hoagii* (ATCC 7005), *C. striatum* (ATCC 6949), and *C. murisepticum* (ATCC 21374).

These strains were cultivated at 37 C for 24 or 48 h on nutrient agar medium, with the exception of *C. diphtheriae*, *C. bovis*, and *C. pyogenes*, which were cultivated on nutrient agar containing 5% calf serum.

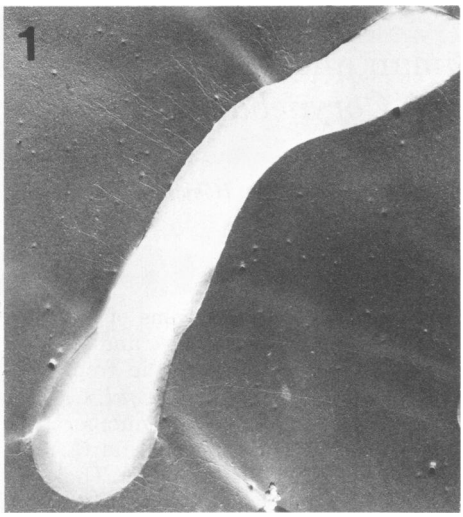
Pili were examined with an electron microscope. Samples were prepared as described previously (15). Bacteria grown on the agar medium were suspended in distilled water, mounted on the collodion grids, and, after reducing the excess amount of the material by absorption with filter paper, were shadowed with palladium. Bacteria suspended in distilled water and fixed in final 0.5% osmium tetroxide overnight at 4 C were, after washing by centrifugation, also examined. In some cases, particularly when those strains possessing only a few pili were examined, bacterial cells grown on collodion membrane combined with a film of agar medium (14) were shadowed. For the negative stain, grown bacteria were suspended in distilled water and mounted on carbon-coated collodion grids and, after reducing the excess amount of the material by absorption with filter paper, were stained with 2% phosphotungstic acid, which was adjusted with 1 N potassium hydroxide to pH 7.2. Preparations were examined in an HU-12A electron microscope (Hitachi Co., Tokyo). The proportion of piliated bacterial cells was counted after random electron microscopic examination of 100 to 250 bacterial cells in each strain.

The ability of bacteria for agglutination of sheep erythrocytes was tested according to a previously described method (9).

Pili were detected in all 11 strains. No essential differences were found between the features of pili of the fixed and unfixed bacteria.

Bacterial cells of *C. kutscheri*, *C. diphtheriae*, and *C. pseudodiphtheriticum* possessed a fairly large number of pili, ranging from dozens to more than a hundred (Fig. 1 and 2). Almost all the bacterial cells of these strains possessed pili (Table 1). After washing with distilled water by centrifugation, the pili apparently remained unchanged. The pili were usually not very long but were slightly longer than the width of the bacterial cell. Bundles of pili somewhat similar to those of *C. renale* type II (15) were found in these strains.

C. equi, *C. hoagii*, *C. xerosis*, and *C. py-*



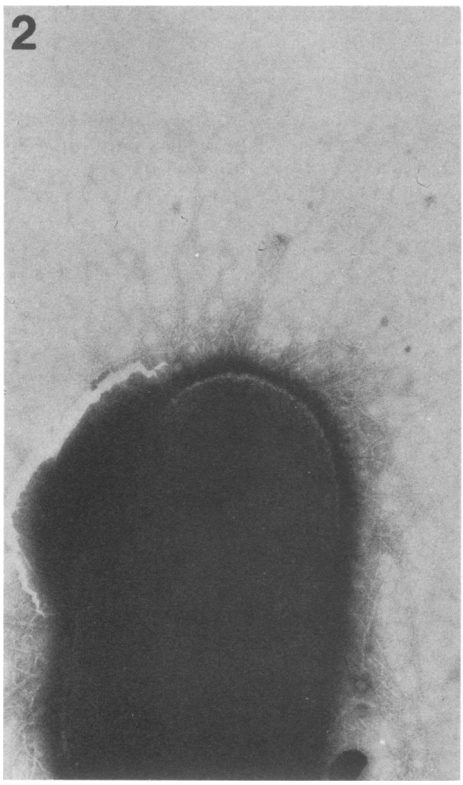


FIG. 1. *C. diphtheriae* (ATCC 19409). ×12,000.
FIG. 2. *C. kutscheri* (ATCC 15677). Negative stain. ×45,000.

ogenes possessed a smaller number of pili. From 10 to 27% of the bacterial cells of these strains were piliated (Table 1). The number of pili per cell was small, only several per cell. The length of the pili was slightly longer than those of the strains of the first group of species and often exceeded the length of the bacterial cell. Bundles of pili were also found. *C. murisepticum*, which tended to make cell aggregates as it grew, possessed similar pili.

The bacterial cell population possessing pili was only 0.5 to 3% in *C. bovis*, *C. pseudotuberculosis*, and *C. striatum*. The number of pili per bacterial cell was small, and at times a long bundle measuring more than several micrometers in length was the only pili seen.

The scantiness of pili in the second and third groups of species was confirmed by examining the bacterial cells grown on collodion membrane combined with a film of agar medium.

The pili are apparently not rigid (Fig. 2). The diameter of pili was estimated from the electron micrographs and also from those of some other strains (Table 1). In examinations thus far, the diameter of pili has been found to be within a 2- to 4-nm range, except *C. pseudodiphtheriticum* and *C. equi*, whose pili were slightly wider (4 to 6 nm in diameter). The internal structure of the pili was not clear.

Agglutination of intact and trypsinized sheep erythrocytes was also examined. Only *C. diphtheriae* weakly agglutinated, and other strains did not agglutinate the sheep erythrocytes. *C. pyogenes* could not be examined, since it showed hemolysis.

It was shown in the present study that 11 species of human and animal parasites and pathogens of genus *Corynebacterium* possessed pili. The presence of the pili in many species of genus *Corynebacterium* may be taxonomically important.

The percentage of piliated bacterial cells and the number of pili per bacterial cell were different among these species. *C. kutscheri*, *C. diphtheriae*, and *C. pseudodiphtheriticum* possessed a fairly large number of pili in almost all of the bacterial cells; on the other hand, other species possessed a small number of pili in only a limited number of bacterial cells.

The characteristics of the pili seemed to be similar to those of *C. renale* pili, in that they were not rigid in appearance, had a tendency to form bundles, and, with the exception of *C. diphtheriae*, did not adhere to the intact sheep erythrocytes. These characteristics contrasted with those of the pili of gram-negative bacteria. The diameter of the pili, 2.5 to 6 nm, did not differ from the pili (common pili) of gram-negative bacteria (2, 5).

The adherence of gram-negative bacteria to sheep erythrocytes and animal cells appears to be due to the pili present (4–8). The pili of *C.*

TABLE 1. *Some features of pili of species of the genus Corynebacterium*

Species	ATCC strain no.	% Piliated cells[a]	No. of pili/ bacterial cell[b]	Length of pili (μm)	Diam of pili[c] (nm)	Bundle formation of pili[d]
C. kutscheri	15677	99	L	0.2–0.9	2–4	(+)
C. diphtheriae	19409	91	L	0.2–1.3	2.5–3.5	+
C. pseudodiphtheriticum	10700	100	L	0.2–1.3	4–5.5	(+)
C. equi	6939	27	S	0.4–2	4–6	+
C. hoagii	7005	27	S	0.5–2.5	2.5–3.5	+
C. xerosis	373	13	S	0.6–3	3–4	(+)
C. pyogenes	19411	10	S	0.2–0.4	2.5–3.5	+
C. murisepticum	21374	37	S	0.4–2	2.5–3.5	(+)
C. bovis	7715	3	S	–10[e]	ND	+
C. pseudotuberculosis	19410	2	S	0.7–2	ND	(+)
C. striatum	6949	0.5	S	0.3–1	ND	+

[a] Bacterial cells ranging from 100 to 250 were randomly examined electron microscopically.
[b] L, Large (from dozens to more than a hundred); S, small (less than 10).
[c] Measured from the negatively stained preparation. ND, Not determined.
[d] +, Frequent; (+), less frequent.
[e] Only long bundles measuring more than several micrometers were seen.

renale also appeared to adhere to the trypsinized sheep erythrocytes (9) and cultured cells (10). In the present study only *C. diphtheriae* agglutinated with both intact and trypsinized sheep erythrocytes. The biological significance and the nature of the pili found in the species of human and animal parasites and pathogens of genus *Corynebacterium* are future subjects for study.

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