JOURNAL OF BACTERIOLOGY, Oct. 1988, p. 4960–4962
0021-9193/88/104960-03$02.00/0

Vol. 170, No. 10

Fine Structures of the Capsules of *Klebsiella pneumoniae* and *Escherichia coli* K1

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Received 16 February 1988/Accepted 8 July 1988

The fine structures of the capsules of *Klebsiella pneumoniae* and *Escherichia coli* were determined by the rapid-freezing technique. The capsular layer was seen as a densely packed accumulation of fine fibers. The thickness of the capsule was approximately 160 nm in *K. pneumoniae* and less than 10 nm in *E. coli* K1. Two layers were observed in the *Klebsiella* capsule in which the arrangements of the fibers were different. The inner layer of the capsule was formed by a palisade of thick and dense bundles of the fibers standing at right angles on the surface of the outer membrane. In the outer layer these thick bundles of fibers loosened into fine fibers which spread over the bacterial surface, forming a fine network structure.

The bacterial species that cause invasive infection usually have a capsule as a virulence factor (4, 6, 9, 10, 12). The capsule is said to act as an antiphagocytic factor or to give serum-resistance characteristics to the bacteria. The detailed mechanisms of this action are not known yet, though much information about the capsules is accumulating from chemical, physiological, and immunological studies (7, 10, 14, 16). We assumed that this ambiguity about the role of the capsule in infection was due largely to insufficient knowledge about the functional structures of the capsules. The electron microscope, which has made possible great progress in our knowledge about the morphology of the bacterial cell, has been absolutely useless for examining capsular structures. This is due mostly to the very fragile nature of the bacterial capsule. During the processing for electron microscopy, major parts of the capsular structure are destroyed. Several efforts have been made to observe the fine structures of the capsules by the conventional chemical fixation method, and the structures have been demonstrated only in bacteria expressing relatively thick capsules under careful fixations (3, 9). Recently, however, a milder fixation method has been developed and applied to the observation of bacterial cell structures. This method has dramatically improved the preservation of the fine structures of bacterial cells (2, 5, 15). With this technique we have demonstrated the presence of a very thin capsule of *Vibrio vulnificus* (2). In this communication we describe the results of using this technique to reveal the fine structures of the capsules of *Klebsiella pneumoniae* and *Escherichia coli*.

The bacterial strains used were a capsulated strain, K, of *K. pneumoniae* and a K1-positive strain of *E. coli*. The strain of *K. pneumoniae* was obtained from our culture stock, and that of *E. coli* was from R. Sakazaki of The National Institute of Health, Tokyo, Japan. The presence of the capsule of *K. pneumoniae* was revealed by the Hiss capsular staining method. This strain is highly virulent to mice. Less than 10 CFU of bacteria injected peritoneally is sufficient to kill the animals. The strain of *E. coli* is of O2:K1:H2 serotype. The capsule, K1 antigen, of this strain was not visible under the light microscope after capsular staining. These bacteria were cultured in nutrient broth or on nutrient agar plates at 37°C. For electron microscopy, bacteria cultured on nutrient agar plates were collected by scraping the lawn of the bacteria on the plates into a small test tube containing nutrient broth. The cells were then centrifuged at 1,000 × *g* for 30 min. The bacteria in the pellet were mixed with melted agar (48°C) and processed for rapid freezing as described elsewhere (1, 15). The rapid freezing was done with a metal contact-type device manufactured by Eiko Engineering Co., Ltd., Tokyo, Japan, and liquid nitrogen was used as a cryogen. Substitution fixation was carried out for 24 h in dry ice and acetone with osmium tetraoxide dissolved at 1% in 100% acetone. The specimen was embedded in Spurr epoxy resin (11). Thin sections were cut with a diamond knife, stained with uranyl acetate and lead citrate, and examined in a JEM 100C electron microscope at 80 kV.

The thin-sectioned profiles of a whole cell of strain K of *K. pneumoniae* and the K1-positive strain of *E. coli* are shown in Fig. 1 and 2, respectively. The outer surfaces of the bacterial cells were covered with a layer filled with fibrous material. This layer definitely represents the capsular layer of these bacteria, since no such structure was seen on the cells of a noncapsulated mutant of strain K or on the K antigen-negative *E. coli* strains. The thickness of the layer was approximately 160 nm in *K. pneumoniae* and 10 nm in *E. coli*. The capsule of strain K was seen to consist of a heavily packed accumulation of fine fibers which represented a polymer of capsular polysaccharide. The electron micrograph (Fig. 1) revealed that the capsule layer has two layers, an inner layer and an outer layer. The fibers were arranged differently in these layers. In the inner half, the fibers formed a dense layer which probably consists of an assembly of thick bundles, which were seen to be standing at right angles to the surface of the outer membrane. In contrast, the outer half of the capsule consisted of a net-like assembly of the fine fibers. Thick bundles of fibers were rarely seen in this layer. The direction of the arrangement of the fibers was random, and some of the fibers were running parallel to the cell surface. These morphological observations suggested that the surfaces of the bacteria were covered with a fine net of capsular polysaccharide which was derived from thick bundles of the polysaccharides in the inner layer. Such two-layered structures of the capsule have also been seen in thin sections obtained from chemically fixed specimens (3, 9), but the more delicate structures were able to be demonstrated by the freezing method. This structure seems to be favorable for the protection of the bacterial cells from attack by enemies in the environment. In contrast to the capsule of *K. pneumo-*

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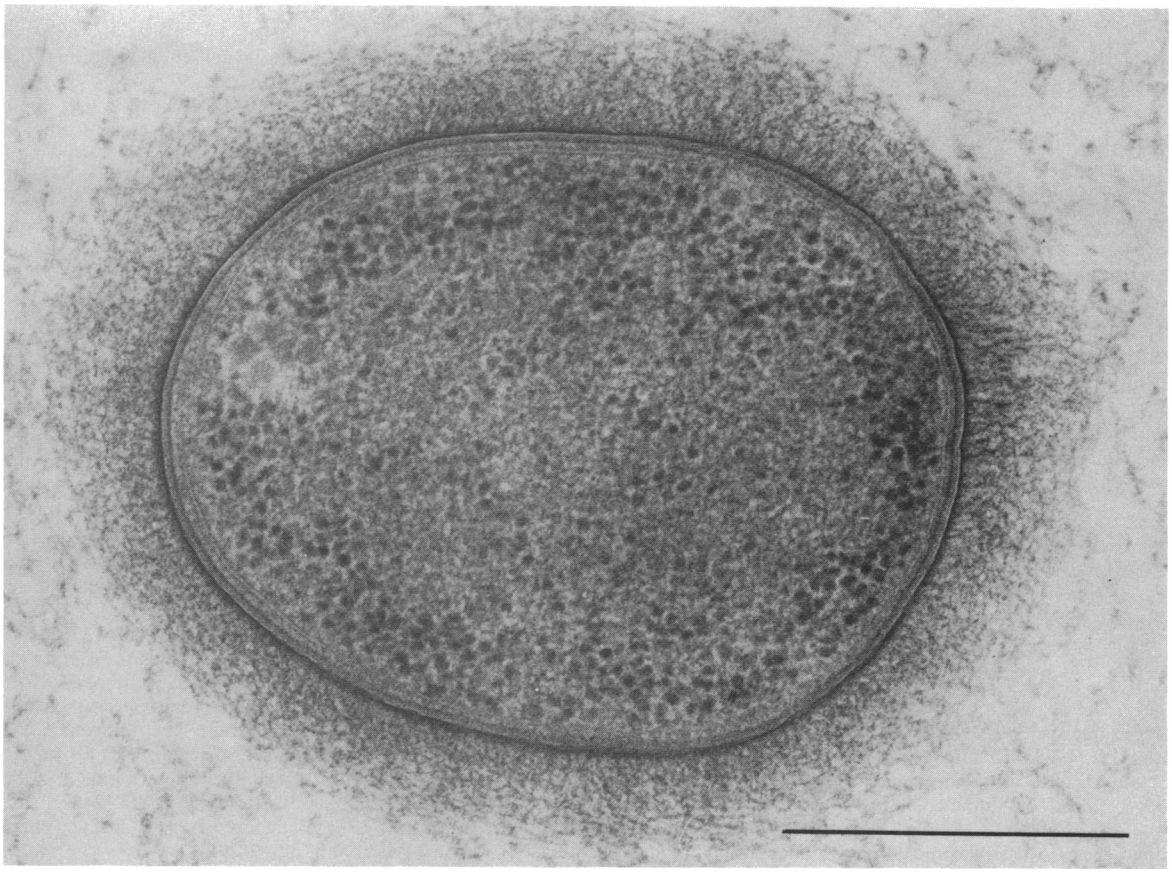

FIG. 1. Thin section of *K. pneumoniae*. The bacterial surface is seen to be covered with a thick layer of the capsule filled with fine fibers. The capsular layer can be separated into two layers, the inner and the outer layers. The arrangement of the fibers is different in these two layers. Bar, 0.5 μm.

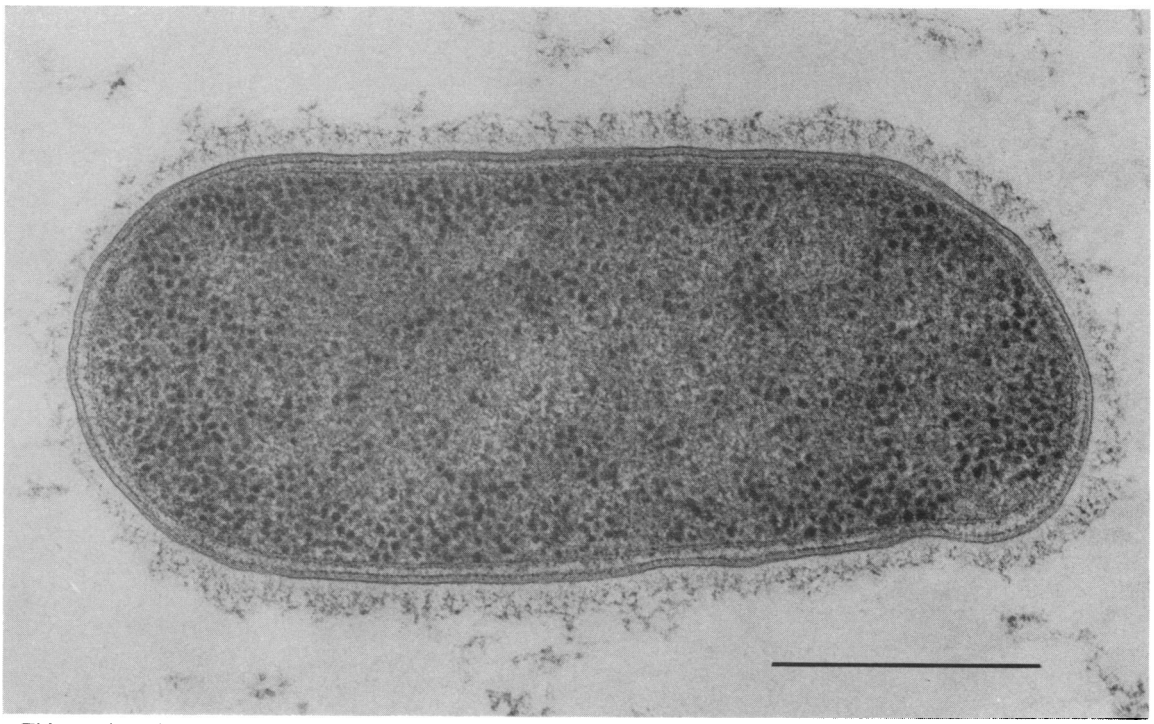

FIG. 2. Thin section of K1-positive *E. coli*. The capsular layer is thin and less dense than that of *K. pneumoniae*; however, the two-layered structure can be seen in the capsule. Bar, 0.5 μm.

niae, that of the K1-positive strain of *E. coli* was very thin and less dense. The capsule was less than 1/10 as thick as that of the *Klebsiella* strain. However, a two-layered arrangement of capsular fibers was also seen in this capsule. At the outer layer of this capsule the arrangement of the fibers was irregular and dense (Fig. 2).

The recent observations on the relationships of the capsular and O-somatic antigens suggest that certain stoichiometric relationships between these two antigens have great significance in relation to the virulence of the organism (7, 8, 13). This could be proved morphologically by using the technique of immunoelectron microscopy and this new fixation method.

We are grateful to Mary Louise Robbins for her kind help in writing the manuscript.

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