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Morphology and Ultrastructure of Staphylococcal L Colonies: Light, Scanning, and Transmission Electron Microscopy

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Scanning electron microscopy utilizing critical point drying was used in parallel with light and transmission electron microscopy to study L colonies produced by a stable L-phase variant of *Staphylococcus aureus* (AH24H).

L-phase variants of bacteria are cell wall-defective forms which are capable of serial replication. On suitable solid media they form distinctive colonies known as L colonies which have slightly elevated, dense central and flat translucent peripheral areas of growth. The individual cells within the colonies vary considerably in size. Large cells grow primarily in the superficial regions of the colonies. Small cells occur within or outside of large cells and constitute the major elements which extend into and multiply within the agar (6).

In the present study, portions of a 24-hr broth culture of a lysostaphin-induced, cell wall-free, stable L-phase variant of *Staphylococcus aureus* (AH24H) were inoculated onto plates of hypertonic agar medium (5, 12-14). After incubation periods of from 6 hr to 5 days, colony-containing agar blocks were removed for study by light microscopy (LM), scanning electron microscopy (SEM), and transmission electron microscopy (TEM).

With LM, L colonies stained by Dienes method (7) or studied unstained by phase contrast microscopy consisted of small clumps of cells by 6 hr. By 12 to 24 hr, their central regions became rather amorphous, whereas their peripheral growth retained its cellular structure and contained many large cells.

With SEM, colonies fixed overnight in a solution containing 2% glutaraldehyde and 5% NaCl, which was buffered to *p*H 7.2 with potassium phosphate buffer and air dried as previously described (5, 13), had a gross morphology which corresponded to that observed with LM. They were difficult to study at high magnification, however, because the individual cells were indistinct and tolerated the primary electron beam poorly. When colonies were similarly fixed but then were treated with a supersaturated solution of uranyl acetate, rinsed with water, dehydrated with increasing concentrations of ethanol, placed in amyl acetate, and dried in a DCP-1 critical point dryer (Denton Vacuum, Inc., Cherry Hill, N. J.) with carbon dioxide as the transitional fluid, the individual cells within the colonies and the fibrillar structure of the agar matrix in which they were embedded were well preserved. Young colonies consisted of spheres of varying diameters (Fig. 1A and B). With continued incubation, the central regions tended to collapse, whereas the peripheral regions contained intact cells (Fig. 1C and D). Some colonies were completely covered by the agar (Fig. 2).

For TEM, glutaraldehyde-fixed colonies were dehydrated in increasing concentrations of ethanol, placed in propylene oxide, embedded in epoxy resin, vertically sectioned, and stained with uranyl acetate and lead hydroxide. Sections through the central portions of well-developed L colonies showed that the cells above the original plane of the agar surface were spherical (Fig. 3A and B), whereas those deep within the agar were pleomorphic (Fig. 3C). In the peripheral portions of the L colonies, the cellular morphology was similar, although there were fewer disintegrated large cells (Fig. 4). At high magnification, intact cells had an ultrastructure similar to that observed in previous studies of L colonies (2-4, 8, 9, 11). They had a limiting unit membrane and contained variable quantities of dense cytoplasmic material. Mesosomes, septa, and organized nuclear material were not seen. Small, spherical, densely packed cells without

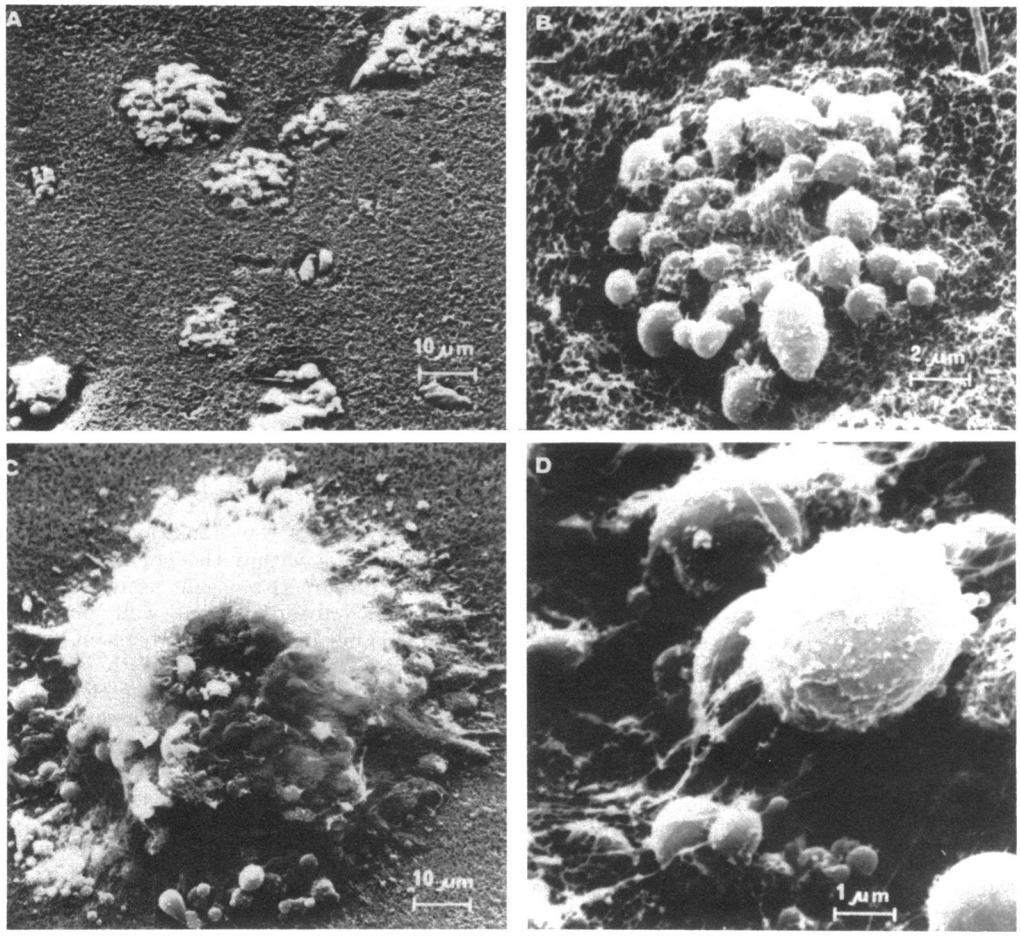

FIG. 1. *Scanning electron microscopy photomicrographs of staphylococcal L colonies. A, B, Six-hr colonies; C, D, 18-hr colony.*

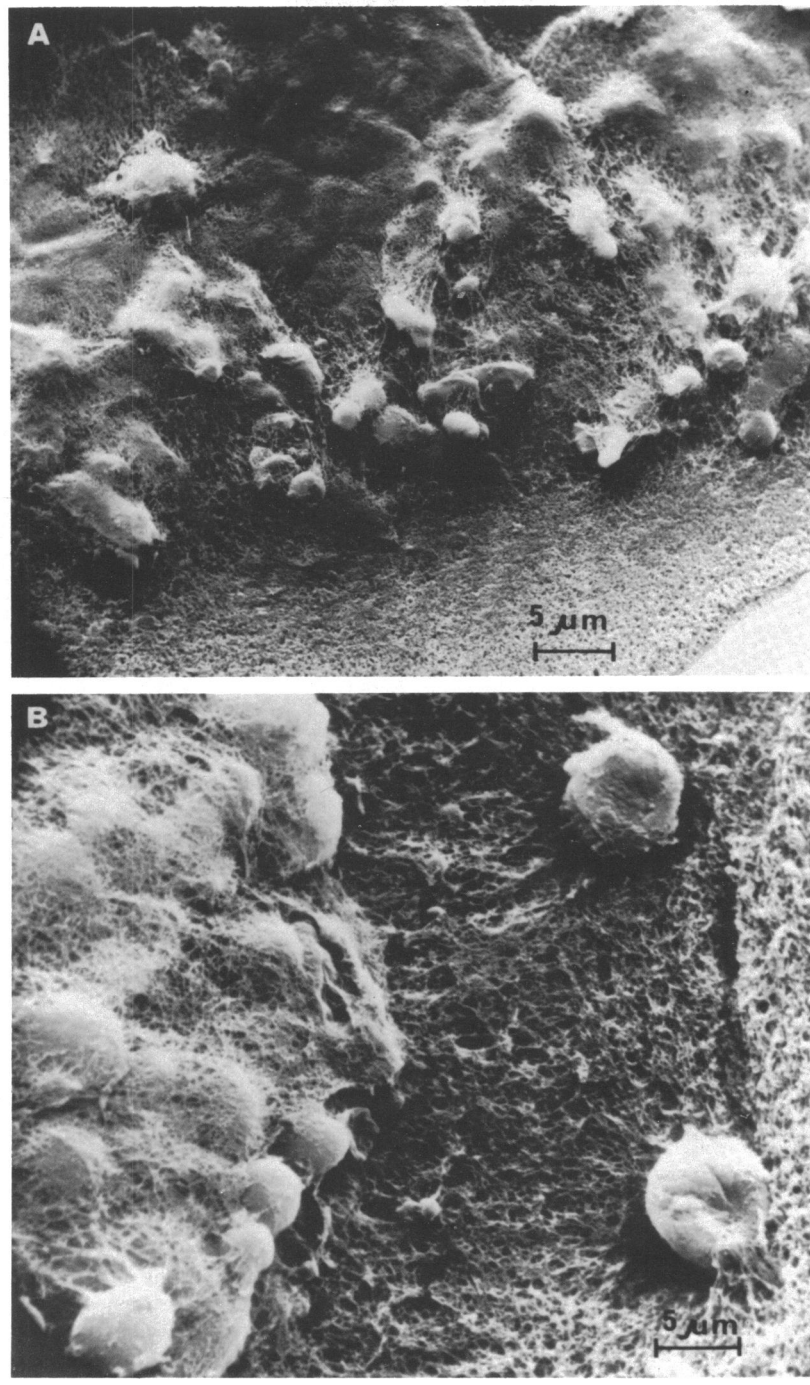

FIG. 2. *Scanning electron microscopy photomicrographs of a 2-day staphylococcal L colony which is covered with agar. A, Tangential view; B, perpendicular view. Isolated large cells which did not multiply are near the margin of the colony.*

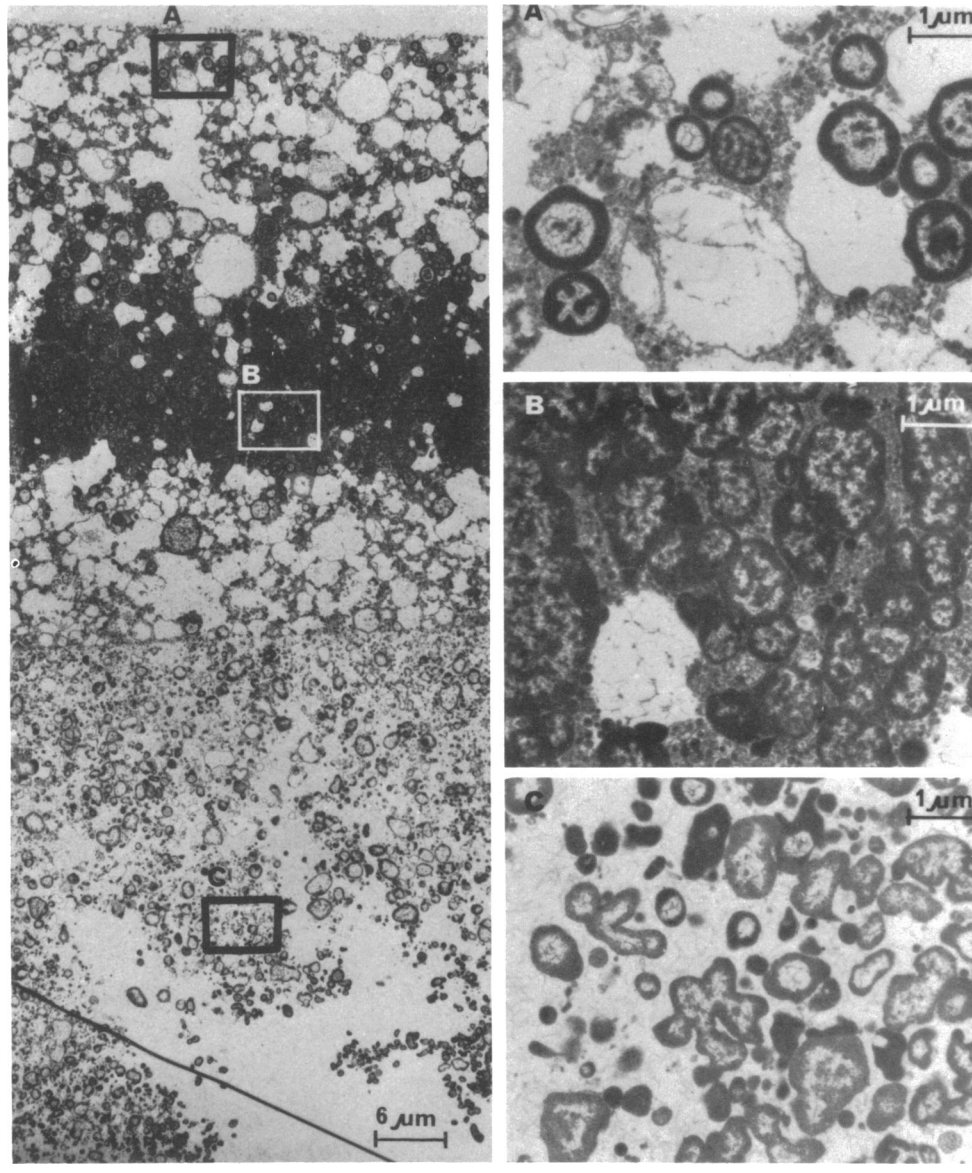

FIG. 3. *Transmission electron microscopy photomicrographs of a vertical section through the central region of a 5-day staphylococcal L colony. A, Superficial region; B, region just above the plane of the original agar surface; C, region deep within the agar.*

apparent nuclear material, which have been previously defined as elementary corpuscles (2), were observed, but morphological evidence of their derivation from the larger forms could not be appreciated. Small cells within large cells, as previously described (1, 10, 15) in broth cultures of L-phase variants, likewise were not observed.

By all methods of microscopy, the staphylococcal L colonies were observed to develop in intimate association with the microstructure of the agar medium. The individual cells within the L colonies were basically spheres whose distribution by size occurred in random fashion, although those deep within the agar were distorted by their physical environment. Elongated and filamentous forms were not observed, and there was no morphological evidence that replication occurred by a complicated life cycle. Differences in morphology between the central and peripheral areas of the colonies seemed to be related to differences in

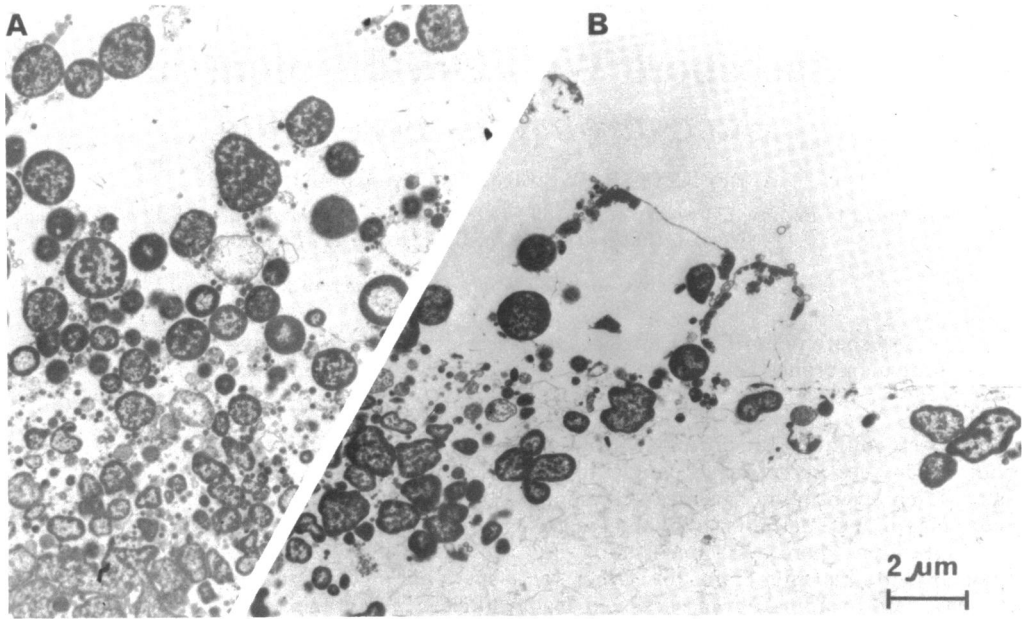

FIG. 4. *Transmission electron microscopy photomicrograph of a vertical section through the peripheral region (A) and the margin (B) of a 5-day staphylococcal L colony.*

age; the dense central areas had a greater depth and contained a relatively larger number of disintegrated large cells.

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