The Ultrastructure of the Almond Leaf Scorch Bacterium with Special Reference to Topography of the Cell Wall

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Accepted for publication 5 August 1975.

ABSTRACT

The ultrastructure of a bacterium occurring in xylem tissue of leaves of almond (*Prunus amygdalus*) displaying typical leaf-scorch (ALS) symptoms were studied *in situ* by electron microscopy. Intracytoplasmic organelles were observed: ribosomes, DNA-like strands, a nuclear region; and occasionally, electron-dense granules and inclusion bodies. The bacterial wall profile consisted of a markedly rippled or ridged outer trilaminar (dense-light-dense) membrane (OM) and a cytoplasmic membrane (CM). Of the two opaque layers of the OM, the inner appeared more electron-dense than the outer. No distinguishable dense intermediate (peptidoglycan) or R layer was present in the periplasmic space. The ALS organism apparently multiplies by binary fission. Extracellular strands and spherical particles were seen in a matrix of low electron-density among the bacteria. A tuft of microfibrils or lotussy was occasionally noted on a few cells.

Prominent bands or ridges on the surface of the OM account for the rippled appearance of the bacterial cell wall. The ridges, separated by furrows or depressions, are randomly or somewhat annularly oriented and appear thicker, wavier in outline, and more closely spaced near the poles. A reconstructed general view of the surface of the cell is shown in sequential sections. Similarity in topography of the cell wall may be supportive evidence for relatedness of the bacteria of grape Pierce's disease, phony peach disease, and ALS.

Phytopathology 66:147-151.

Additional key words: xylem pathogen, *Draeculacephala minerva,* grape Pierce's disease, phony peach disease.

In recent years a disease causing leaf scorching symptoms has appeared in almond orchards in California (10). A bacterium was found in xylem tissue of diseased leaves and was transmitted by the leafhopper, *Draeculacephala minerva* Ball (7, 8). The almond leaf-scorch (ALS) organism possesses very similar ultrastructure to that of the Pierce's disease bacterium (9) and the organism associated with phony disease of peach (13). The three bacteria are xylem-limited and leafhopper vectored. We examined the ultrastructure of the ALS bacterium and the topography of its cell wall *in situ* by electron microscopy. Studies on the etiology of ALS and the transmission of the disease by grafting as well as by a leafhopper vector are reported in another paper (7).

MATERIALS AND METHODS.—The midrib portions from leaves of almond (*Prunus amygdalus* Batsch 'Long IXL') with typical symptoms of ALS were excised and prepared for electron microscopy as described previously (5). Leaf pieces 1-2 mm in size were immersed in 2.5% glutaraldehyde for 2 hours, washed in 0.1 M sodium phosphate buffer, pH 7.0-7.2, and postfixed in OsO_4 for 4 hours or longer at 4 C. After dehydration in an acetone series for 4 hours, the pieces were embedded in Spurr's medium (15). Ultrathin sections, 60-90 nm, were cut with a diamond knife, mounted on copper grids, stained with uranyl acetate and lead citrate and examined with an electron microscope (RCA EMU 3H) operating at 50 kv.

RESULTS AND DISCUSSION.—*General morphology.*—Numerous bacteria occurred in the lumina of xylem vessels from the affected almond leaves. More bacteria occurred in some vessels than in others. Invaded vessels were usually in groups and up to 15% of the vessels were infected in the most severely diseased samples. In longitudinal median section the ALS bacterium is typically elongate with rounded ends (Fig. 1). Some ends were tapered, and these were noted more frequently than could be attributed to the orientation of the section to the knife. In cross-section the cell is spherical or ovoid. The average diameter of 542 cells was 0.4 μm; the maximum length in median longitudinal section, 1.9 μm. The length of the cells is somewhat less than that of Pierce's disease and phony peach bacteria (5, 9, 13).

In longitudinal view, especially, the bacterium shows an unusual multilayered cell wall (Fig. 1, 2). The wall profile consists of an outer trilaminar (dense-light-dense membrane (OM), ridged, furrowed, or rippled in varying degrees; and a relatively smooth "unit" membrane, the cytoplasmic membrane (CM). The inner layer of the OM appears more electron-dense than the outer layer (Fig. 2). The OM and CM are separated by an electron-lucent zone or periplasmic space in which no distinguishable dense intermediate (peptidoglycan) or R layer (3, 4) is seen. The OM including the periplasmic space may be up to 50 nm thick. The thickness of the ridges of the OM and the plane of section account for variation in measurements of the wall.

The cell contents of the ALS organism are generally similar to the commonly described components of the bacterial cells: ribosomes, DNA-like strands, and a nuclear region (Fig. 1). In occasional cells electron-dense granules, 40-50 nm in diameter (Fig. 3, 4), and membranous inclusions or vesicles (Fig. 3) are found. The electron-dense granules, comparable to those described as polyglucoside granules (14), and also seen in Pierce's

disease bacteria (unpublished data), are present in two morphological forms (Fig. 3, 4). In one form they present a shrivelled appearance with the degenerated or depleted contents shrunk away from the periphery of a sac-like enclosure (Fig. 3). In another form, presumably in a biosynthetically active cell, the granules appear "full," larger, and more uniform (Fig. 4). A mesosome-like inclusion (Fig. 3) seems to be formed from the invagination of the CM. Internally it has a discrete structure with strand-like ramifications.

That the ALS bacterium multiplies by binary fission is suggested by the median constrictions observed in some cells (Fig. 5). The cell wall and CM appear to invaginate synchronously in a partitioning process presumably resulting in two daughter cells.

Extra-cellular "debris".—A matrix or "debris" of low electron density is present among the bacterial cells in the lumina of xylem vessels. In the matrix two kinds of morphological entities are detected: extracellular strands that appear made up of subunits and spherical particles of a size similar to the subunits of the strands (Fig. 8, 10, 11). The particles, about 30 nm in diameter, may have originated from the strands. Occasionally the strands are seen attached to the OM of the wall of a bacterial cell (Fig. 8). The two entities are similar to those observed in grape affected by Pierce's disease (unpublished data) and peach affected by phony disease (13).

We observed an electron-lucent zone in the matrix (Fig. 6) around some cells of the ALS bacterium. A similar zone or "halo" was noted around cells of the Pierce's disease bacterium (9). A possibly comparable electron-lucent zone noted in another microorganism, *E. coli*, was considered lipoproteinaceous (12).

We occasionally noted an electron-dense capsular matrix, also found in the phony peach organism (13), that appeared as a tuft of osmiophilic lines or "micro-fibrils" radiating from the periphery of one end of a few bacterial cells (Fig. 7). There was no "halo" around such cells. A very similar structure, termed lotussy, was described recently on a microorganism in human gingival plaque (6).

Topography of the cell.—Striking surface views of the OM are seen in oblique longitudinal sections containing the cell wall (Fig. 10, 11). The unusual ultrastructural topography of the wall results from convolutions of the OM seen as bands or ridges, separated by furrows, randomly or somewhat annularly oriented and parallel to the short axis of the cell, especially near its poles. The bands or ridges at the poles appear to be thicker, more wavy in outline and more closely spaced than those away from the poles (Fig. 10, 11). The width of the ridges varies from 45-75 nm. The furrows or depressions between the ridges are 10-30 nm wide.

Reconstructed general views of the topography of the wall from six different cells are presented in sequential sections (Fig. 9, 12). The bands or ridges (Fig. 9) in longitudinal sections appear to be more closely spaced as the wall is sectioned further inward and eventually they blend together at the bottom of the furrows or the base of the ridges before the cytoplasmic contents are exposed. In transverse sections at the polar region (Fig. 12) the overlapping of ridges is evident particularly in Fig. 12a. The variation in electron-density in Figs. 12b and 12c is due to exposure of the cytoplasm.

Unusual surface structure of the OM of the wall has been noted also on other bacteria (3, 4). The wall of *Veillonella* (2) has convolutions or rod-shaped elements that appear to be randomly distributed on the OM of the diplococcal cells. The fine structure of the wall surface of *Methanospirillum hungatii* shows minute, parallel "stacked bands" (16). The bands are uniform and orderly spaced on the OM of the cell wall. Another type of surface structure, described on *Spirillum serpens* from isolated cell wall fragments, consists of a hexagonal array of structural subunits (11). Many medically important species of several genera have wall surface structure similar to that of *S. serpens* (3, 4).

The similarity in architecture of the wall surface of the bacteria associated with Pierce's disease, phony peach, and ALS, in view of other evidence, suggests to us that these organisms may be related. The topographical features of these bacteria may have taxonomic significance. Additional evidence for relatedness is that they are xylem-limited and have at least some leafhopper vectors in common. ALS and Pierce's disease have similar leaf symptoms in their primary hosts and the symptoms can be induced by cross inoculation (7). Their causal agents are reported to be Gram-positive (1, 7). Although the Gram-stain property of the phony peach organism has not been established, morphologically and ultrastructurally this organism (13) resembles closely those from grape and almond.

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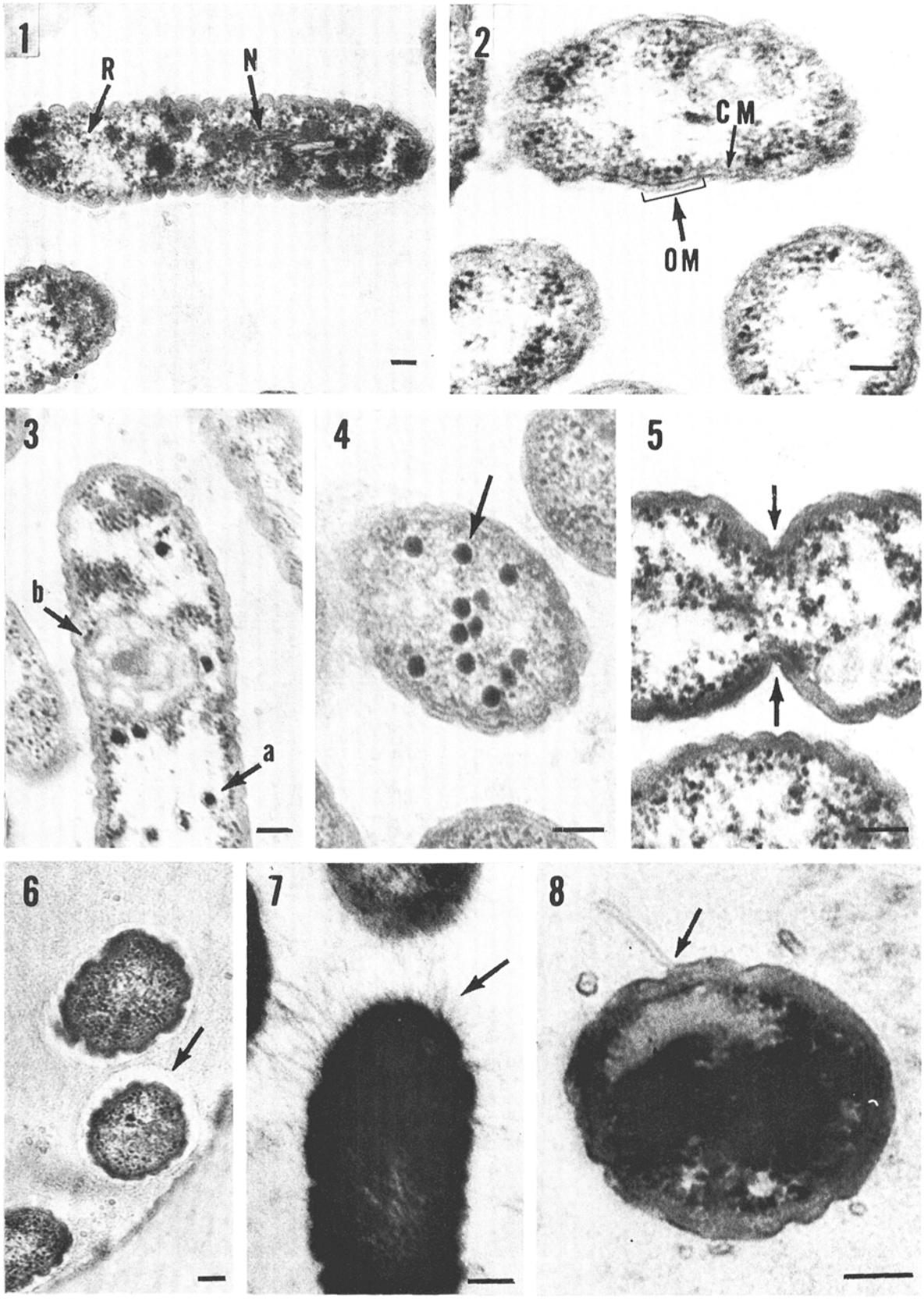
Fig. 1-8. Electron micrographs of almond leaf scorch (ALS) bacteria in xylem vessels of mid-veins of diseased almond leaf. Scale bars = 0.1 μm. **1)** A typical rod-shaped bacterium in longitudinal view showing rounded ends and a strikingly rippled or ridged wall profile. Note ribosomes (R) and nuclear region (N). × 43,000. **2)** Bacteria in longitudinal and cross sections. Note the more electron-dense inner layer of the outer membrane (OM) of the cell wall (arrow) and the cytoplasmic membrane (CM) (arrow). × 78,000. **3)** A portion of a bacterial cell containing (a) many degenerated or depleted electron-dense granules, presumably polyglucoside, and (b) an inclusion body (mesosome-like) that has a central discrete structure and strand-like ramifications. × 60,000. **4)** Transverse section of an organism containing electron-dense granules (arrow) similar to those in Fig. 3, except these granules are fully developed, and more regular in appearance. × 81,000. **5)** Longitudinal view of a portion of a bacterial cell undergoing binary fission. Note the median constrictions (arrows) and the synchronous invagination of the cell wall and cytoplasmic membrane. × 79,000. **6)** A "halo" (arrow) surrounding an individual cell. × 44,000. **7)** A portion of a bacterial cell in longitudinal section displaying numerous osmiophilic lines or "micro-fibrils", which are very similar to a structure termed "lotussy", radiating from the cell wall. × 74,000. **8)** A bacterial cell in transverse section with a strand attached to the OM (arrow). Note also the detached or "free" particles in the vicinity of the cell. × 112,000.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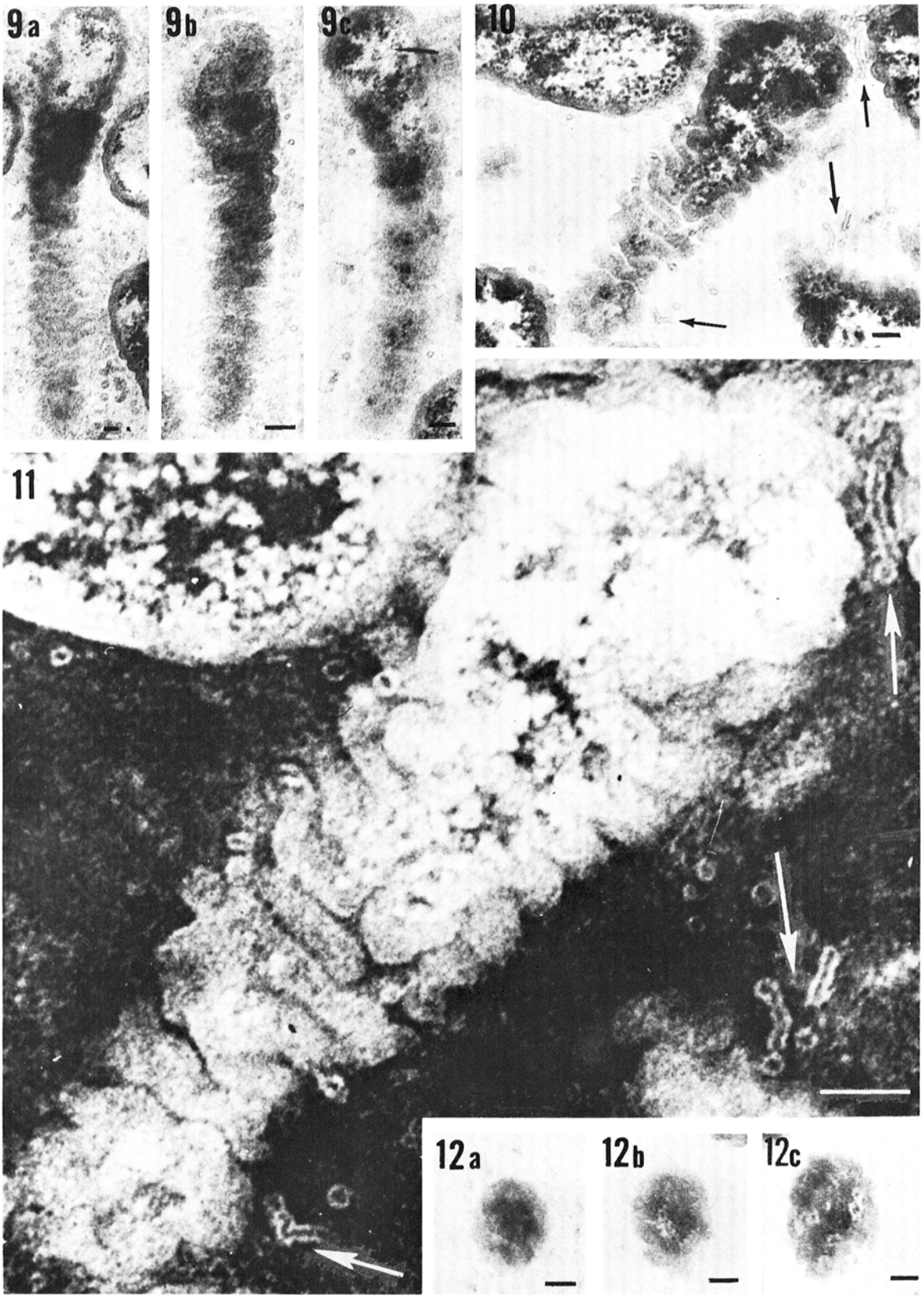
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Fig. 9-12. Surface views (longitudinal and transverse) of ALS bacteria in the lumina of vessels of diseased almond leaf. **9a, b, c)** Three sequential longitudinal sections depicting the topography of the bacterial cell wall. Sections a, b, and c represent progressively greater depth of cut into the walls of cells; and in c, ridges appear to blend together, just before the cytoplasm is exposed. a, × 41,000; b, × 52,000; and c, × 45,000. (10, 11) Longitudinal (oblique) section of a bacterium showing, in great detail, prominent bands or ridges of varying dimensions (45-75 nm wide) that result in the rippled appearance of the cell wall. The ridges, separated by furrows, randomly or somewhat annularly oriented, appear to be thicker, wavier, and more closely spaced near the poles. Note the detached particles and strands composed of subunits (arrows). **10)** × 53,000. **11)** A highly magnified view (negative image) of Fig. 10, not to be confused with negative staining. × 151,000. **12a, b, c)** Three sequential transverse sections at the polar region of cells. Note overlapping of ridges (12a). More cytoplasmic contents are visible as the cells are sectioned inward (12b, 12c). a, × 54,000; b and c, × 50,000.

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