NOTES

Parasporal Bodies of *Bacillus laterosporus* Sporangia

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Intact colonies of *Bacillus laterosporus* examined by thin-section transmission electron microscopy revealed sporangia in various stages of development and degeneration as the endospores matured. The sporangia formed a surface layer of hexagonally arranged subunits. The variety of parasporal bodies raised questions of developmental and ecologic utility.

*B. laterosporus* spp. are frequently studied for their developmental properties and their ability to produce protein parasporal bodies (PBs). Some of these proteins are entomocidal and thus used as biological controls (5). A distinct PB made up of approximately 20 layers and with an ultrastructure similar to that of the spore coat has been described for *Bacillus laterosporus* (8). The stacked layers adhere to and cover 25 to 50% of the spore surface with a final three-dimensional shape resembling a canoe or the keel of a ship. There may be more than one of the lamellar PBs on a spore (Fig. 1A and E). The protein of the PB readily reacts with electron microscopic stains, giving a characteristic density.

The purpose of this investigation was to describe two additional PBs and call attention to an outer surface layer of the sporangium. An unidentified strain of *B. laterosporus*, provided by the Centers for Disease Control, Atlanta, Ga., was streaked on standard nutrient agar medium (Difco Laboratories, Detroit, Mich.) and incubated at 20 to 25°C for 48 to 72 h. Additional glucose of 0.1 to 0.5% gave slightly larger colonies with no apparent differences in sporogenesis or microscopic properties. Typical colonies with diameters of 3 to 4 mm or cut wedges of larger growths were processed intact so that intercellular orientation, associations, and cell products could be noted in situ. Colonies were fixed in 0.1 M PIPES [piperazine-N,N'-bis(2-ethanesulfonic acid)]-buffered (pH 7.0) 5% glutaraldehyde solution for 1 h at room temperature. This was followed by a second fixation in a 0.1 M PIPES-buffered (pH 7.0) 2% osmium tetroxide solution for 1 h at 4°C and en bloc staining in 1% uranyl acetate for 1 h at 4°C. Dehydration was done in a graded acetone series, and then embedding was done with Spurr low-viscosity resin (13). The final embedment with 100% plastic was done with 6 h of centrifugation at 1,200 × g to ensure infiltration of the spores. Sections were cut with a diamond knife, mounted on Formvar-coated, single-slot grids, stained with uranyl acetate in 50% ethanol and Reynolds lead citrate (11), and examined with a Phillips EM 201 electron microscope at an accelerating voltage of 60 kV.

Thin sections through the colony revealed ultrastructural properties of cells in different stages of development. Most of the cells of the central region were in the mature spor stage. The spore, along with the lamellar PB, nearly filled the sporangium. The PB, made up of sequentially smaller layers, distended the sporangial wall (Fig. 1A–E). A visual estimate of this PB placed its volume near that of the spore. Much of the usually ribosomal-textured cytoplasm was replaced by fibrous structure apparently continuous with the PB surface (Fig. 1E). Cytoplasmic residue remained in the sporangial periphery (Fig. 1B). There was no evidence of a loose outer layer of spore coat, which is recognized as exosporium and common to *Bacillus anthracis* (9), *Bacillus subtilis*, *Bacillus thuringiensis*, *Bacillus megaterium*, and *Bacillus cereus* (F. A. Montaldi, Ed.D. dissertation, University of Georgia, Athens, 1970), nor was there a discernible membrane associated with the spore or PB.

A second PB, globular to angular in shape, appeared in *B. laterosporus* sporangia concomitant with the lamellar PB (Fig. 1A, C, and D and 2C). It was 100 to 200 nm in diameter, homogeneous in electron density, and without direct ties to the spore or the lamellar PB.

A third PB, striated with alternating parallel light and dark bands, appeared within the cell envelope complex (Fig. 1A–C and E). With its regular periodicity, the sectioned image resembled a streamer (Fig. 2A–C) or a picket fence (Fig. 1A and B). The three 70-nm serial sections supported a three-dimensional image of a rod with a diameter of at least 200 nm (Fig. 2D–F). The dark bands appeared to be layers or plates of 3-nm thickness and 5-nm spacing made up of dense material. Fibers emanated from both sides of the plates in brushlike arrays. The brushes overlapped to form the lighter bands as they appeared in intact sporangia (Fig. 1A). With sporangial autolysis, there was disassembly and separation of the brushes, leaving a space between the brush fibers where the light bands once were (Fig. 2B and C).

In cross section, the gram-variable envelope of the sporangium during later stages of development appeared to be made up of three layers. The outer layer and sometimes the inner layer had regular interconnections, imparting a zipper-like image (Fig. 1B). With tangential sections grazed through a plane of the envelope, there was a regular lattice substructure of 20-nm repeating units in hexagonal order (Fig. 2C–F) appearing to conform to those making up the surface layer found on several gram-positive and -negative species (3, 12). Preliminary attempts to demonstrate these layers with freeze-fracture, with and without etching, were unsuccessful. The characterization of the outer layer as an S layer awaits further investigation.

While the parasporal proteins of several *Bacillus* spp. have...
FIG. 1. Electron micrographs of thin sections of *B. laterosporus*. (A) Sporangia with mature spores (s) and lamellar (l) and striated (st) PBs. Bar, 0.5 μm. (B) Sporangium with the lamellar (l) and striated (st) PBs. Three-layered envelope is shown (en). Bar, 0.25 μm. (C) Sporangium with homogeneous PB (ho) and oblique section of striated PB (st). Bar, 0.5 μm. (D) Sporangium with lamellar (l) and homogeneous (ho) PBs. Bar, 0.25 μm. (E) Sporangium with two lamellar (l) and two striated (st) PBs and numerous flagella (f). Bar, 0.5 μm.
FIG. 2. Electron micrographs of *B. laterosporus* sporangia. (A) Striated PB (st) revealed by relatively shallow section. Bar, 0.25 µm. (B) Separating brushes (br). Bar, 0.25 µm. (C) Shallow sections through two sporangia, showing separated brushes (br), a homogeneous PB (ho), and hexagonally ordered surface subunits (su). Bar, 0.25 µm. (D–F) Serial sections to show three-dimensional form of striated PB (st) in end portion of a sporangium. Sections revealed hexagonal pattern of surface layer subunits (su). Bar, 0.25 µm.
received much attention for their application as entomocidal agents (2, 4, 5). B. laterosporus has only recently been reported as a pathogen to mosquito and black fly larvae (7) and white grubs (6). In addition, B. laterosporus has been reported to produce spergusalin, which with its 15-deoxy derivative has been found to be prophylactic and curative for lupus erythematosus-like lesions (10). Spergusalin has been shown to be an antitumor antibiotic in transplantable leukemias in mice (14). Although antibiotics are generally referred to as secondary metabolites produced and released by cells approaching the dormant stage (1), there is as yet no evidence linking spergusalin or other toxins of B. laterosporus to sporangial activities.

The question of ecological advantage arises for any phenotypic property and especially for a presumably energy-expensive process such as the production of parasporal proteins or other products by a cell whose sole function is to produce a dormant spore and thereafter self-digest. The production of PBs may not necessarily be adaptive. The organisms may have several plasmids, self-replicating and transient DNA molecules not vital to the general survival of the species, that encode for the parasporal proteins (e.g., B. thuringiensis [15]). Since the cell is not in its normal vegetative state, production of proteins may be unchecked at this time, and coats and PBs could simply be the result of spontaneous crystallization or deposition of these excess proteins (15). However, the PBs may be adaptive if their proteins are antimicrobial or entomocidal or of nutritional value during germination.

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LITERATURE CITED