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Electron Microscope Studies of Conditional Spore Cortexless Mutants of *Bacillus sphaericus*

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Spore cortex of conditional cortexless mutants of *Bacillus sphaericus* 9602 was not detectable by electron microscopy unless the medium was supplemented with *meso*-α,ϵ-diaminopimelic acid during sporulation. Other spore structures appeared normal. Spore shape was quite irregular in the absence of *meso*-α,ϵ-diaminopimelic acid.

Biochemical analysis of spores of conditional cortexless mutants isolated from *Bacillus sphaericus* 9602 showed that cortex content in the mutant spores was less than 5% of the wild-type cortex content (3). To confirm this result, spores of conditional cortexless mutants produced with or without DL-Dap (mixture of *meso*, LL, and DD-diaminopimelic acid, Sigma Chemical Co., St. Louis, Mo.) during sporulation were examined by electron microscopy.

Wild type and group I, II, and III of L-lysine-requiring mutants of *B. sphaericus* were grown at 32°C for 18 h in SP medium supplemented with 100 g of L-lysine per ml, as described previously (3). DL-Dap (1 mg/ml) was added as indicated. Electron microscopic analysis was carried out after making ultrathin sections (Fig. 1 to 4).

Wild-type spores are round and have a thick, white layer of cortex (Fig. 1). Inner forespore membrane, germ cell wall, outer forespore membrane, inner spore coat, lamellar midcoat, outer spore coat, and exosporium were clearly observed, as recently reported (2). A group I L-lysine-requiring mutant (mutant 20-1) that has a defect in *meso*-Dap decarboxylase produces refractile and round-shaped spores when grown in the presence of L-lysine (3) and is identical to the wild type (Fig. 2). Cortex synthesis was normal.

A group II L-lysine-requiring mutant (mutant 32-3) that is defective in *meso*-Dap synthesis but can make dipicolinic acid (DPA) produces nonrefractile and oval-shaped spores in the presence of L-lysine but in the absence of DL-Dap during sporulation (3). As shown in Fig. 3A, B, and C, spores had an irregular shape and cortex was not visible. Other structures, germ cell wall, spore coats, and exosporium seemed to be normal. The irregularity of shape confirms the idea that cortex strongly contributes to maintenance of the rigid structure. Pleated ("crenulated") structure of spore coats is clearly seen in cross-sections (Fig. 3b). Probably a normal amount of spore coat is accumulated around the spore, but the space to be covered by it is much smaller than in the normal spore, owing to the absence of the space normally filled with cortex. In the presence of DL-Dap in the medium, the group II mutant produces refractile and cortex-containing spores (3). Under these conditions, mutant spores are indistinquishable from wild-type spores by electron microscopy (Fig. 3D-F).

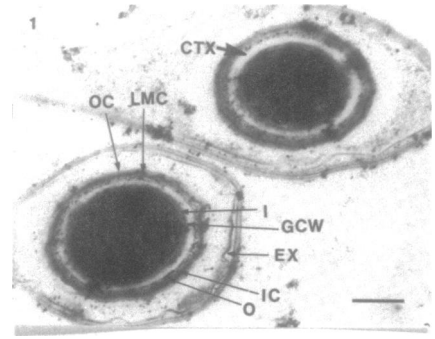

FIG. 1. *Thin section of B. sphaericus wild-type sporulating cells. I, inner forespore membrane; GCW, germ cell wall; CTX, cortex; O, outer forespore membrane; IC, inner coat; LMC, lamellar midcoat; OC, outer coat; EX, exosporium. Bar = 0.25 μm.*

A group III L-lysine-requiring mutant (mutant 21-1) that has defects in both *meso*-Dap and dipicolinic acid synthesis also produces nonrefractile and oval-shaped spores in the absence of DL-Dap during sporulation (3). By elec-

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tron microscopy, only cortex is missing from the mutant spore, and other spore structures are normal (Fig. 4A). In the presence of DL-Dap in the medium, the spores contain a normal cortex (Fig. 4B).

These results clearly indicated that cortex content measured by muramic lactam content truly reflects the real content of cortex in the spore. Furthermore, the mutants isolated as conditional cortexless mutants are only defective in cortex synthesis, and synthesis of other spore structures is quite normal. It is especially interesting that the mutant spores contain a normal amount of spore coat in the absence of DL-Dap during sporulation. When DL-Dap concentration in the medium was varied, there were some conditions under which the spores were xylene resistant but still octanol sensitive (4). These data, coupled with the above results which show the presence of complete spore coats in the spores produced without DL-Dap in the medium, indicate that spore coats are not the only factor required for octanol resistance. The presence of cortex and the consequent production of a dehydrated spore cytoplasm may be a major factor in octanol resistance. This result

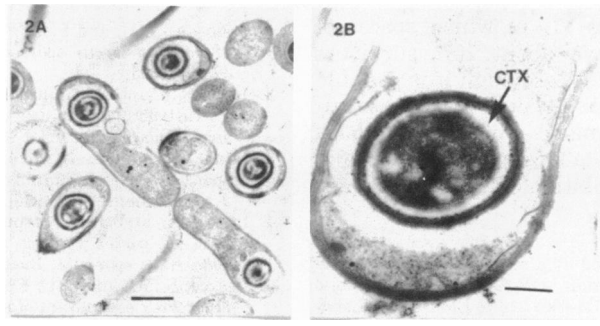

FIG. 2. *Thin section of a group I L-lysine requiring mutant (20-1). (A) Bar = 1.0 μm; (B) bar = 0.25 μm.*

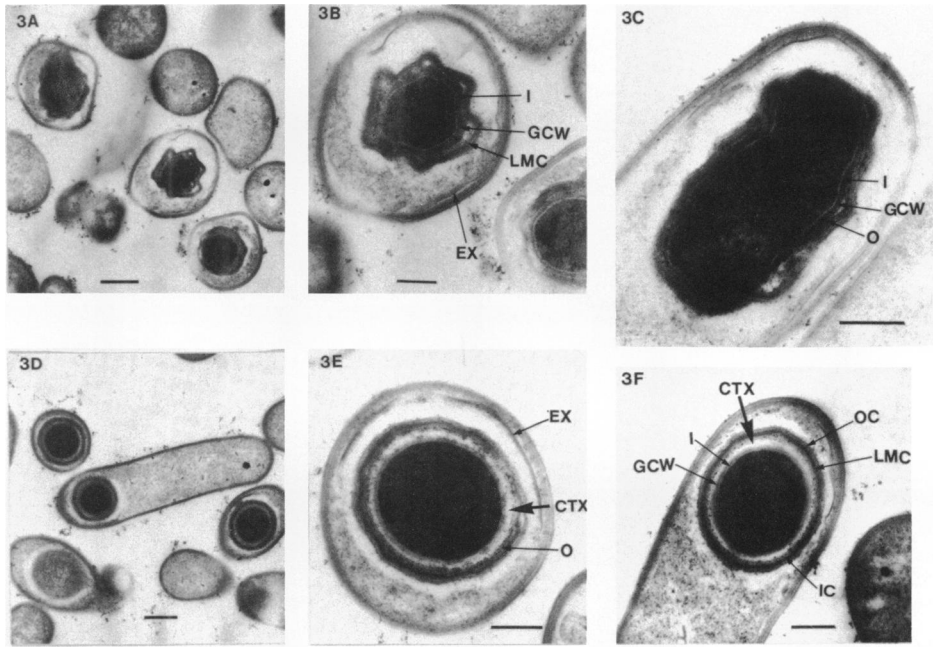

FIG. 3. *Thin section of a group II L-lysine-requiring mutant (32-3). (A-C) Spores produced without DL-Dap. (D-F) Spores produced with DL-Dap (1 mg/ml). (A) Bar = 0.5 μm, (B) bar = 0.25 μm, (C) bar = 0.25 μm, (D) bar = 0.5 μm, (E) bar = 0.25 μm, (F) bar = 0.25 μm.*

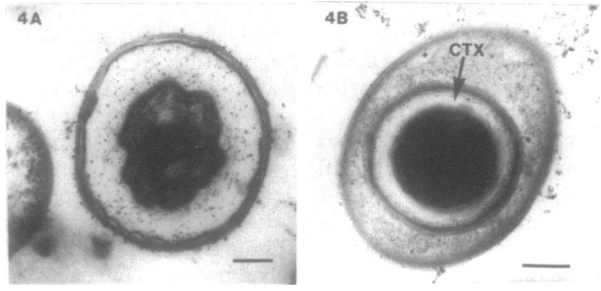

FIG. 4. *Thin section of a group III L-lysine-requiring mutant (21-1). (A) Spores produced without DL-Dap. Bar = 0.25 μm; (B) spores produced with DL-Dap (1 mg/ml). Bar = 0.25 μm.*

is supported by the recent report of spore coat mutants of *B. cereus* (1) in which spores of coatless mutants gave about 10% survivors after octanol treatment (although it is possible that the mutation was "leaky" and the survivors were a few organisms which formed both cortex and coat). This laboratory had also previously reported the isolation of a cortexless mutant of *B. cereus* (5).

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