JOURNAL OF BACTERIOLOGY, Nov., 1965
Vol. 90, No. 5

Multiple Septation in Variants of *Bacillus cereus*

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Received for publication 20 June 1965

ABSTRACT

REMSEN, C. C. (Syracuse University, Syracuse, N.Y.), AND D. G. LUNDGREN. Multiple septation in variants of *Bacillus cereus*. J. Bacteriol. **90**:1426–1431. 1965.—Abnormal nonsporulating cells observed in cultures containing predominantly endospores were examined in an electron microscope. One or more septa were seen which consisted of cell-wall material between two unit membranes. Numerous membrane swirls were often seen associated with the septa. Some of the reported structural features of the variant cells were similar to those published for *Bacillus cereus* var. *alesti* and *B. subtilis*; however, the majority of the cells observed in this work were multiseptated.

While studying the fine structure of *Bacillus cereus*, Pfister and Lundgren (1964) observed both membrane and cell-wall invaginations distinct from normal transverse septum and forespore formation. Such structures were generally absent from cells forming normal spores, and, in the culture system used, represented a few per cent of the cell population. Abnormal cells contained as many as eight invaginations at either pole. A preliminary report of such septa formation has been given (Pfister, Lundgren, and Merrick, Bacteriol. Proc., p. 26, 1964).

Young (1964) recently reported a "walling-off" of polar compartments in *B. cereus* var. *alesti*; she referred to these as "pseudoforespores," and has given evidence that these structures contain a full set of genes and are viable. In our strain of *B. cereus*, single polar-septation was observed, but most abnormal cells showed multiple septation.

MATERIALS AND METHODS

B. cereus ATCC 4342 was examined after growth at 37 C in either a glucose-glutamate-glycine-salts medium or in the same medium containing less glucose (1 g per liter); the culture apparatus and conditions were as described by Cooney and Lundgren (1962). Cell samples were removed at various time intervals, rapidly frozen in a Dry Ice bath, and stored until prepared for sectioning.

Fixation, embedding, sectioning, and electron microscopy were done by the procedures reported by Pfister and Lundgren (1964).

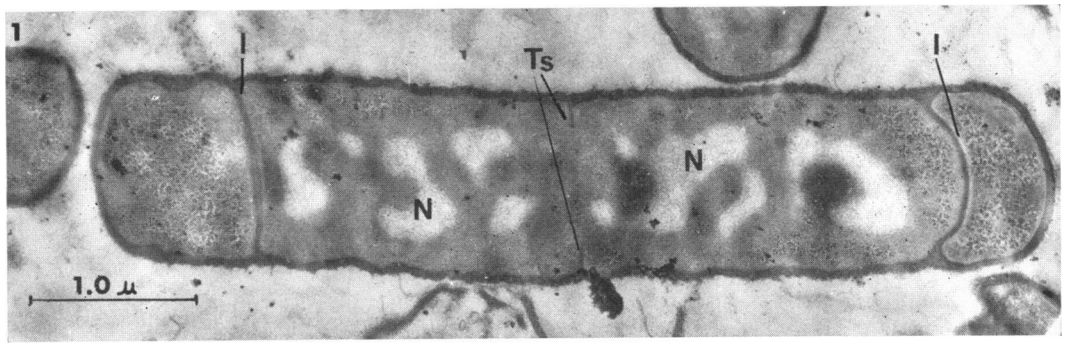

FIG. 1. *Thin section of Bacillus cereus. Cell contains polar septa (I) and a partially formed transverse septum (Ts). Nuclear material (N) appears in the central portion of the cell.* × *24,150.*

RESULTS

During vegetative growth of our strain of *B. cereus*, no morphological abnormalities were

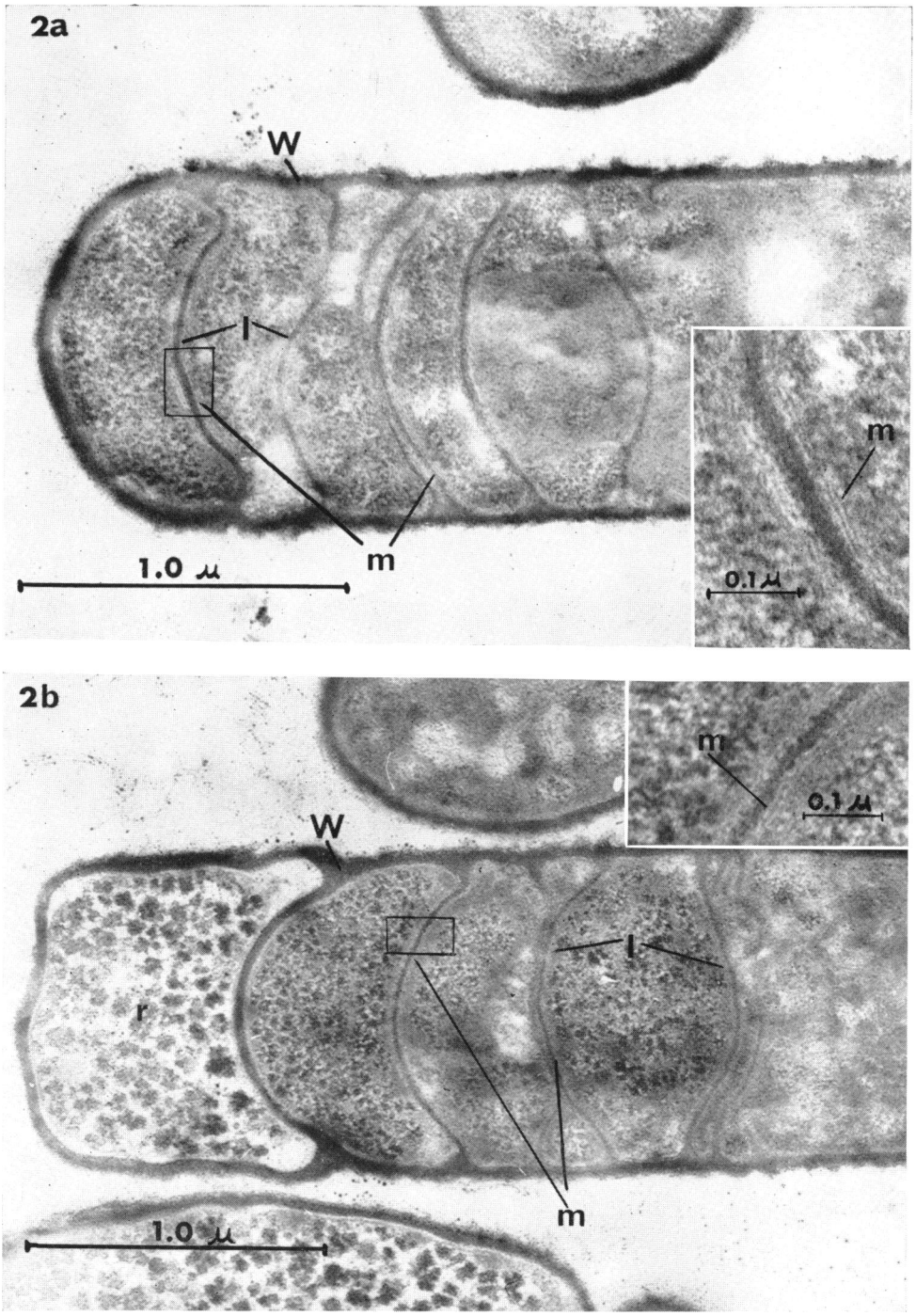

FIG. 2. *Thin section of Bacillus cereus. Cells show the invaginations (septa; I) at one end of the cell. The septa consist of cell-wall material (W) and are bordered by unit membranes (m). Structures believed to be ribosomal clusters (r) are also shown in the squared segment of the cell. (2a) × 45,000. (2b) × 41,500. Inserts show a further enlargement of the membrane (m) as marked by the inked-in square. (2a) × 123,000. (2b) × 110,000.*

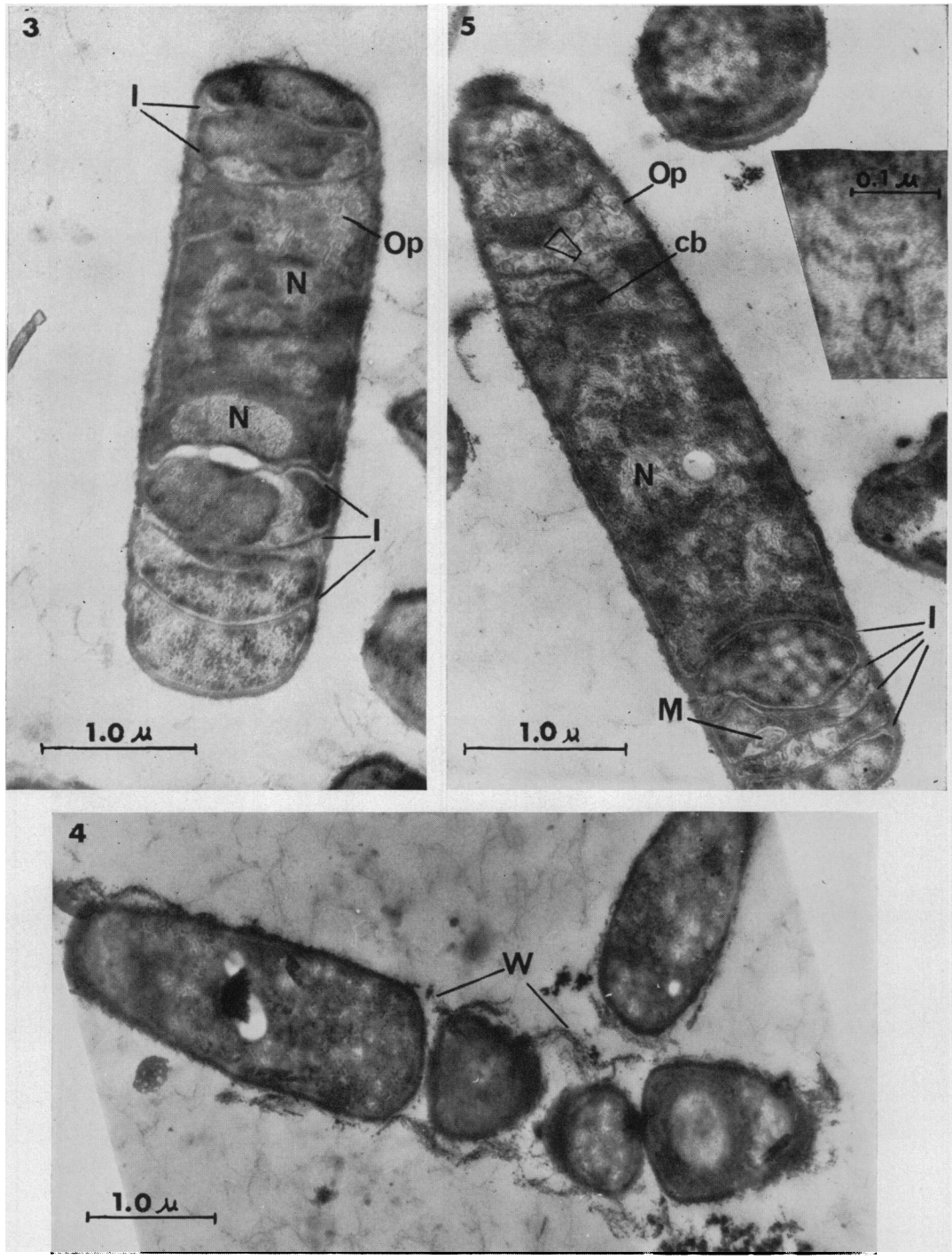

FIG. 3. *Thin section of Bacillus cereus. Cell shows invaginations (septa; I) at either pole with nuclear material (N) found in the central portion of the cell. Intracytoplasmic membrane elements (Op) are seen associated with some septa.* × *23,000.*

FIG. 4. *Thin section of Bacillus cereus. Cell shows the end result of abnormal septation with the release of "discrete" units. The remains of wall material (W) are also visible.* × *18,500.*

FIG. 5. *Thin section of Bacillus cereus. This cell was poststained with lead hydroxide for 45 min and shows intracytoplasmic membrane elements (membrane swirls; Op) as well as a cytoplasmic body (cb). Invaginations (septa; I), nuclear material (N), and a mesosome (M) are also visible. Insert is an enlargement* (× *130,000*) *of the membranous swirls found in the cell, as marked by the square.*

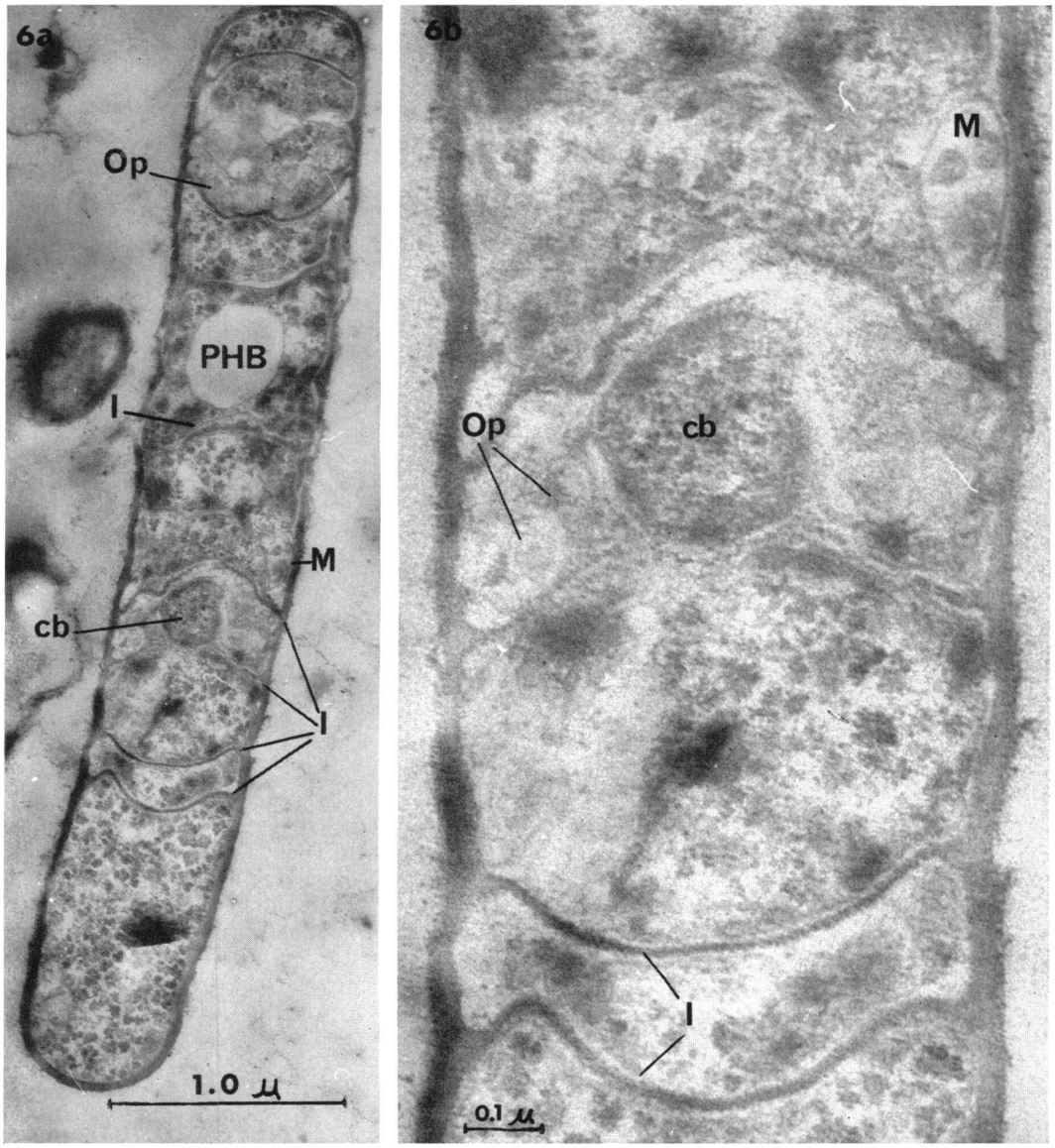

FIG. 6a. *Thin section of Bacillus cereus. The partially lysed variant cell shows invaginations (septa; I), poly-β-hydroxybutyrate (PHB) granule, a cytoplasmic body (cb), a mesosome (M), and membranous swirls (Op).* × *33,500.*

FIG. 6b. *Enlargement of part of Fig. 6a.* × *110,000.*

noted. However, during sporulation variants were noted which, instead of forming the normal forespore membranes, formed double transverse membranes, usually towards the poles, with cell-wall material deposited between the membranes. Figure 1 shows a cell with complete polar septa walling off portions of the cell and a partially formed transverse septum. This cell probably aborted at stage two of sporulation where portions of the axial filament (nucleus) are seen in what would normally become the sporangium (Ryter, 1965). Some cells showed multiple invaginations (septa) mostly towards the poles (Fig. 2a and b), consisting of unit membranes with cell-wall material deposited between them. The inserts in Fig. 2 show an enlargement of a particular area. A square polar segment, as seen in Fig. 2b, was observed in some variants in which cytoplasmic extraction had occurred, revealing ribosome-like clusters similar to those

reported by Pfister and Lundgren (1964). Cell variants which formed multiple invaginations, although present in the same culture, appeared morphologically distinct from cells having the single polar invaginations as shown in Fig. 1.

The presence of chromatinic material in the walled off segments has not been clearly resolved, but nuclear elements were seen in the center portion of the cell (Fig. 1, 3, and 5). Segments are eventually liberated (Fig. 4) when lysis of the cell wall occurs.

Several sections suggested the association of membrane swirls and mesosomes with septa formation. When thin sections of *B. cereus* were poststained with lead hydroxide for longer periods of time, more specific membrane contrast was observed at the expense of the other cellular components (Fig. 5). Numerous membranous swirls (intracytoplasmic membrane elements) within the walled-off segments were readily seen. The insert shows an enlargement of the swirls. The membranous elements are not too unlike the structures associated with septation seen in negatively stained preparations of *Bacillus stearothermophilus* (Abrams, 1965). Figure 6a shows a variant cell with multiple septa and with both intracellular membranous swirls and mesosomes; a membrane-bound storage granule is also evident (Pfister and Lundgren, 1964). An enlargement of parts of this cell is shown in Fig. 6b.

DISCUSSION

Abnormal cells in sporulating cultures or such cells grown as pure cultures have been previously reported in both *B. subtilis* and *B. cereus* var. *alesti*. Ryter, Ionesco, and Schaeffer (1961) showed that, in sporulating cultures of *B. subtilis*, two mutants, $Try^{-}Sp^{-}4$ and $Sp^{-}12$, exhibited abnormal polar septation and lacked the capacity to form spores. Similarly, Young (1964) showed, in *B. cereus* var. *alesti*, pseudoforespores [mutant A(−)3] which were similar cytologically to the $Try^{-}Sp^{-}4$ mutant of *B. subtilis* and to some of the abnormal cells discussed in the present report. The pseudoforespores, like normal forespores, are initially formed by the invagination of the plasma membrane; however, maturation is incomplete and there is deposition of cell-wall material between the layers of the membrane septa. More recently, Ryter (1965) suggested that in *B. subtilis* septation at both poles may be a normal occurrence of sporulation and that during the initial stages only one septum goes on to develop into a spore, with the other inhibited very rapidly. The variants which form two septa contain three nuclei, but are unable to complete sporulation; instead, cell-wall material is deposited between the membranes.

Although some of our abnormal cells demonstrated those morphological features described by Young (1964) and Ryter (1965), cells with multiple septation were seen more frequently. These cells may or may not fall into Young's class of "pseudoforespores." To date, there is no evidence showing that each cell segment contains a complement of deoxyribonucleic acid (DNA). Although possible, a cell containing 10 segments would have to produce 10 copies of its DNA. We did observe some cell fracture after segmentation (Fig. 4), and each segment may be capable of growth.

As an explanation of abnormal septation, Young (1964) suggested that the structural abnormality seen in mutant A(−) 3 is associated with the presence of lysogenic phage. No evidence is available for the involvement of a phage in our system.

Multiple septation may be associated with the role membranes play in nuclear division. Jacob Brenner, and Cuzin (1963) suggested that cell membranes in bacteria play the role of the mitotic apparatus and that during DNA replication new membranes are formed. More recent information reported by Ryter and Landman (1964), Ryter and Jacob (1964), Ryter (1965), and Fitz-James (1965) suggests that there is a close association between the cell nucleus and the cytoplasmic membrane and that often a mesosome is intermediate to both. These authors feel that nuclear replication is triggered at a point of contact with the cell membrane, and that it is coordinated with cellular septation. Thus, if each segment in these abnormal cells is viable, and assuming septation is a result of DNA replication, one explanation may be that there is no longer any control over membrane-associated DNA replication.

Further cytological studies are planned using pure cell lines with multiple septation to learn more about this phenomenon.

ACKNOWLEDGMENT

This investigation was supported by Atomic Energy Commission Contract AT-30-1(2038).

LITERATURE CITED

ABRAMS, D. 1965. Electron microscope observations on intact cells, protoplasts, and the cytoplasmic membrane of *Bacillus stearothermophilus*. J. Bacteriol. **89**:855–873.

COONEY, J. J., AND D. G. LUNDGREN. 1962. Studies of asporogenic mutants of *Bacillus cereus*. Preliminary investigations with calcium and zinc. Can. J. Microbiol. **8**:823–833.

FITZ-JAMES, P. C. 1965. A consideration of bac-

terial membrane as the agent of differentiation. Symp. Soc. Gen. Microbiol. **15**:369–378.

JACOB, F., S. BRENNER, AND F. CUZIN. 1963. On the regulation of DNA replication in bacteria. Cold Spring Harbor Symp. Quant. Biol. **28**: 329–348.

PFISTER, R. M., AND D. G. LUNDGREN. 1964. Electron microscopy of polyribosomes within *Bacillus cereus*. J. Bacteriol. **88**:1119–1129.

RYTER, A. 1965. Étude morphologique de la sporulation de *Bacillus subtilis*. Ann. Inst. Pasteur **108**:40–60.

RYTER, A., H. IONESCO, AND P. SCHAEFFER. 1961. Étude au microscope électronique de mutants asporogene de *Bacillus subtilis*. Compt. Rend. **252**:3657–3677.

RYTER, A., AND F. JACOB. 1964. Étude au microscope électronique de la liaison entre noyau et mésosome chez *Bacillus subtilis*. Ann. Inst. Pasteur **107**:384–400.

RYTER, A., AND O. E. LANDMAN. 1964. Electron microscope study of the relationship between mesosome loss and the stable L state (or protoplast state) in *Bacillus subtilis*. J. Bacteriol. **88**:457–467.

YOUNG, I. E. 1964. Characteristics of an abortively disporic variant of *Bacillus cereus*. J. Bacteriol. **88**:242–254.