Ultrastructure of *Bacillus subtilis* 168 treated with cerulenin

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Abstract

The ultrastructure of *Bacillus subtilis* 168 grown for 1 h during the initial stage of sporulation in the presence of cerulenin was studied. At all concentrations used (5, 15 or 25 μg/ml) the antibiotic caused no damage to the structure of the cell wall, the cytoplasm or the nucleus. The size, structure and frequency of the mesosomes did not change. In some of the cells more than two septa were established even when a forespore was already present. It is possible that cerulenin, which is an inhibitor of fatty acid synthesis, might interfere with the prevention of septum formation in cells carrying a forespore or another septum.

Introduction

The antibiotic cerulenin [(2S), 3R 2,3-epoxy-4-oxo-7,10-dodecadienoylamide] is a specific inhibitor of fatty acid synthesis in cells (Omura, 1976). In studies on the macromolecular synthesis and cell division of *Streptococcus faecalis* it was established that lipid and lipoteichoic acids synthesis, as well as cell number increase, were affected prior to any observable effects on mass increase or DNA, RNA, protein and peptidoglycan synthesis (Carson and Daneo-Moore, 1978)

Higgins *et al.* (1980) studied the effect of cerulenin on the ultrastructure of the same micro-organism and showed that the antibiotic interfered with cell division and septum formation. The authors attributed this action of the drug to its ability to block the synthesis of a lipid-containing inhibitor of autolytic enzyme activity necessary for division. Autolysin activity is presumed to be necessary for controlled cleavage of the mucopeptide polymer surrounding the cell. Amphiphiles such as lipoteichoic acids and cardiolipin were found to inhibit dissolution of walls of several Gram-positive species (Shockman and Barrett, 1983). In several instances, the presence of fatty acid esters was shown to be required for inhibition of autolysin activity. The state of the membrane of *B. subtilis* also proved to be important in its regulation.

Wille *et al.* (1975) established that *B. subtilis* stopped growing when cerulenin was added to the culture medium, but the cells remained completely viable. The antibiotic blocked *de novo* synthesis of fatty acids and lipids. Therefore, it was of interest to study the ultrastructure of *B. subtilis* when the cells were treated with a drug disturbing the synthesis of components involved in the regulation of autolysin activity.

Materials and methods

Growth conditions

Bacillus subtilis (strain 168) was grown in a medium containing peptone (Difco) (1% w/v), yeast extract (Difco) (1% w/v), NaCl (0.5% w/v),

Na_2HPO_4 (0.04% w/v), pH 7.2. Growth proceeded for 12 h at 37°C with aeration, and cerulenin (Makor Chemicals, Ltd, Jerusalem, Israel) dissolved in 0.2 ml of 95% ethanol diluted with warm water (40°C) to a concentration of 1 mg/ml was added to the culture, in final concentrations of 5, 15 or 25 μg/ml. Cultivation continued in the presence of cerulenin for 1 h and the cells were harvested by centrifugation and washed twice in 0.05 M tris-HCl buffer, pH 7.5.

Electron microscopy

Cells of *B. subtilis* 168 were fixed overnight in OsO_4 by the method of Kellenberger and Ryter (1965) and embedded in durcopan. Staining was performed by the method of Reynolds (1963). Thin sections of bacteria were examined using an Opton CM electron microscope.

Results and discussion

Cells of *B. subtilis* 168 grown for 12 h were treated with cerulenin (5, 15 or 25 μg/ml) for 1 h. At all the concentrations used the antibiotic caused no damage to the structure of the cell wall, the cytoplasm or the nucleus (Figures 1a—d). The size, the structure, and the frequency of mesosomes (usually found around the nucleus) did not change during this treatment.

The cells had an appearance, typical for the initial stages of sporulation, *e.g.* they possessed a single elongated nucleus in contact with one or two mesosomes. In the cerulenin-treated bacilli the formation of more than one septum in cells which already carried a septum or a forespore (Figures 2a—d) was observed. These septa were either symmetrically or asymmetrically initiated (Figures 3a and b), and contained cell wall material when completed (Figures 2a—e). Some of the cells had a strange appearance (Figure 2e, 3c and 3d). The effect of the drug was most pronounced when cells were treated with lower concentrations (5 or 15 μg/ml), while with a growth-inhibiting concentration of cerulenin (25 μg/ml) such anomalies were not established (Figure 1e).

As shown by Ryter (1965) when a forespore was formed in *B. subtilis*, a second septum might be initiated, but it rarely came to completion, as its development was quickly inhibited. Ryter *et al.* (1966) have described a mutant of *B. subtilis* which formed two septa. Neither the rarely encountered bacteria from the wild type which possessed two septa, nor the previously mentioned mutant, which was blocked in stage II of sporulation, formed real spores.

Our ultrastructural studies have shown that the cerulenin-treated cells of *B. subtilis* 168 resembled the mutant of sporulation described by Ryter *et al.* (1966), in that it formed more than one septum each of which contained cell wall material. As discussed by Ryter (1965), although a second septum might be initiated in *B. subtilis*, its completion was prevented by unknown factor(s). It seems possible that cerulenin has an effect on these factors either directly by inhibiting their activity, or indirectly by inhibiting the synthesis of lipids

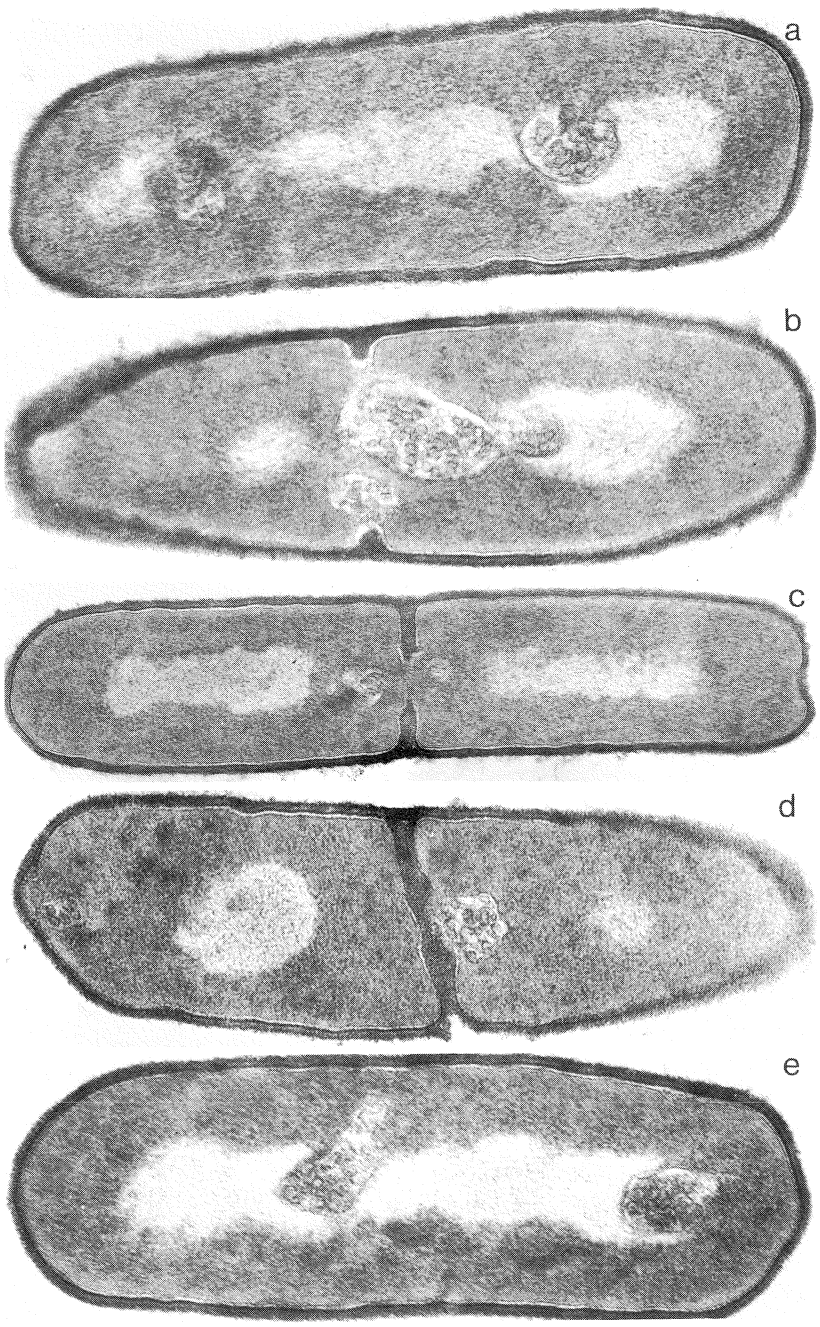

Figure 1a Ultrathin section of *B. subtilis* 168 not treated with cerulenin. x 40,000.
Figure 1b Initiation of a septum in *B. subtilis* 168. x 40,000.
Figure 1c Protrusion of cross wall into the cytoplasm. x 40,000.
Figure 1d Completed septum. x 40,000.
Figure 1e *B. subtilis* treated with 25 μg/ml cerulenin. x 40,000.

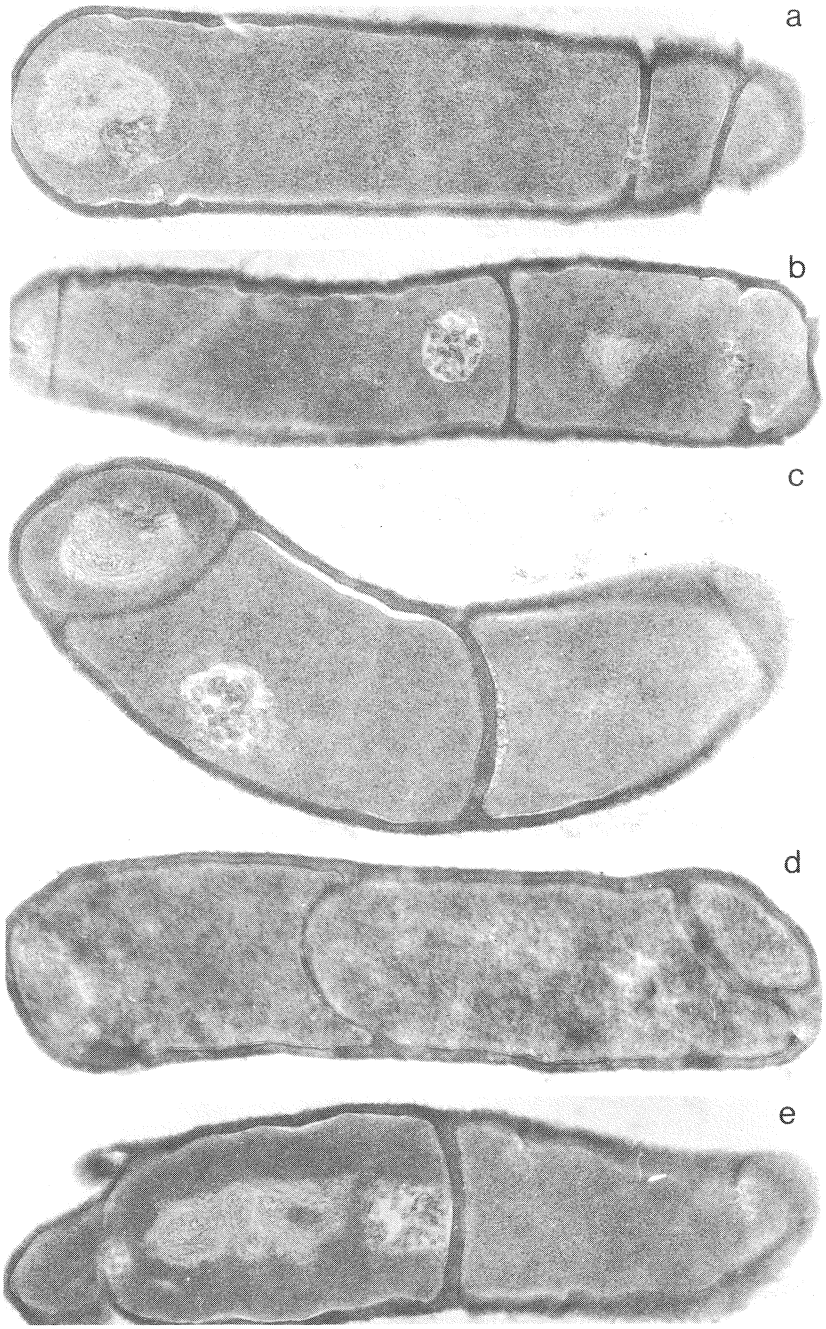

Figure 2 Ultrathin sections of cells of *B. subtilis* 168 treated with cerulenin showing anomalies in septum formation, *i.e.* formation of more than one septum in a cell. The septa contain cell wall material.
Figure 2a A forespore and two septa are formed. x 64,000.
Figure 2b Two septa are formed and a third one is initiated. x 50,400.
Figure 2c Three septa are formed. x 40,000.
Figure 2d Two septa are formed, one of them asymmetrically located. x 64,000.
Figure 2e Two septa are formed. x 40,000.

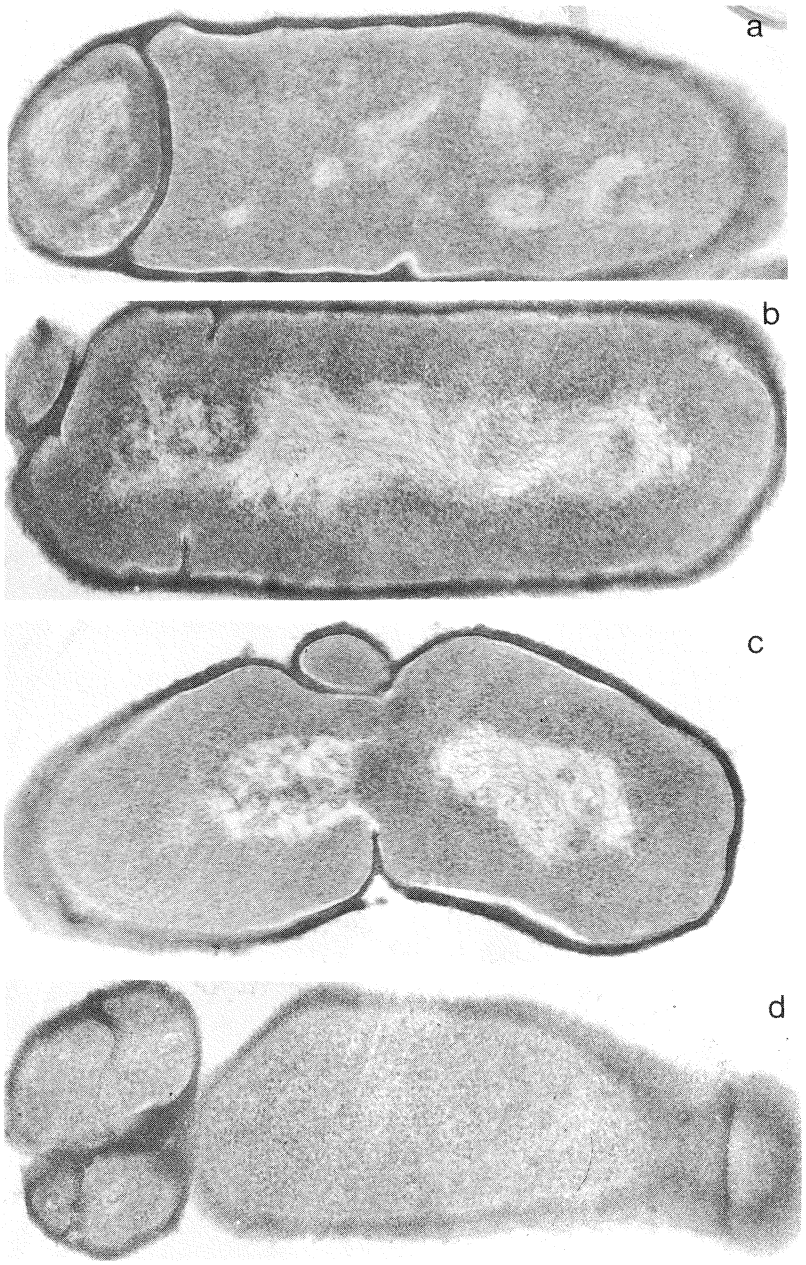

Figure 3 Ultrathin sections of cells of *B. subtilis* 168 treated with cerulenin with deviations in septum formation.
Figure 3a Asymmetrical initiation of septum. x 50,400.
Figure 3b The second septum is initiated very close to the already formed septum. x 50,000.
Figure 3c Unusually located septum. x 50,400.
Figure 3d Formation of several septa in one cell. x 40,000.

and lipoteichoic acids which were found to be involved in the process (Diederick Meyer and Wouters, 1984; Shockman and Barrett, 1983). This allows not only a second septum to be formed in the cell, but also other septa to be initiated and even completed.

References

CARSON D. and Daneo-Moore L. 1978. Effect of cerulenin on *Streptococcus faecalis* macromolecular synthesis and cell division. *J. Bact.* **133** 472–6.

DIEDERICK MEYER P. and Wouters J. T. M. 1984. Effect of lipoteichoic acid on cell wall turnover in *Bacillus subtilis*. *Antonie van Leeuwenhook* **50** 85.

HIGGINS M. L., Carson D. D. and Daneo-Moore L. 1980. Morphological effect of cerulenin treatment on *Streptococcus faecalis* as studied by ultrastructure reconstitution. *J. Bact* **143** 989–94.

ŌMURA S. 1976. The antibiotic cerulenin, a novel tool for biochemistry as an inhibitor of fatty acid synthesis. *Bact. Rev.* **40** 681–97.

REYNOLDS E.S. 1963. The use of lead citrate at high pH as an electron stain in electron microscopy. *J. cell Biol.* **17** 208-12.

RYTER A. 1965. Etude morphologique de la sporulation de *Bacillus subtilis*. *Annls. Inst. Pasteur (Paris)* **108** 40–60.

RYTER A., Schaeffer P. and Ionesco H. 1966. Classification cytologique, par leur stade de blocage, des mutants de sporulation de *Bacillus subtilis Marburg*. *Annls. Inst. Pasteur (Paris)* **110** 305–15.

SHOCKMAN G. D. and Barrett J. F. 1983. Structure, function and assembly of cell walls of Gram-positive bacteria. *A. Rev. Microbiol.* **37** 501–27.

WILLE W., Eisenstadt E. and Willecke K. 1975. Inhibition of *de novo* fatty acid synthesis by the antibiotic cerulenin in *Bacillus subtilis*: effect on citrate-Mg^{2+} transport and synthesis of macromolecules. *Antimicrob. Agents Chemother.* **8** 231–7.

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