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NOTES

Effects of Penicillin G on Mesosome-Like Structures in *Agmenellum quadruplicatum*

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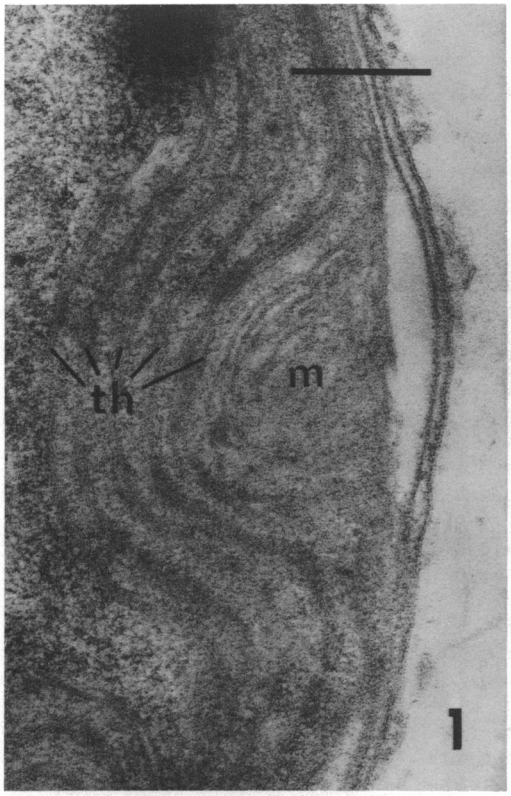
Agmenellum quadruplicatum was treated with lethal doses of penicillin G (15 and 75 U/ml) and then examined by electron microscopy to observe changes that took place before lysis. Treatment for 30 min led to increases in the frequency and size of mesosome-like structures within the cells. There was a direct correlation between the magnitude of these increases and the concentration of penicillin G. The mesosome-like structures often were associated with division septa, and they resembled mesosomes reported in other cyanobacteria. It is suggested that these structures appeared and enlarged because synthesis of membrane material continued after wall (peptidoglycan) synthesis was inhibited by the drug.

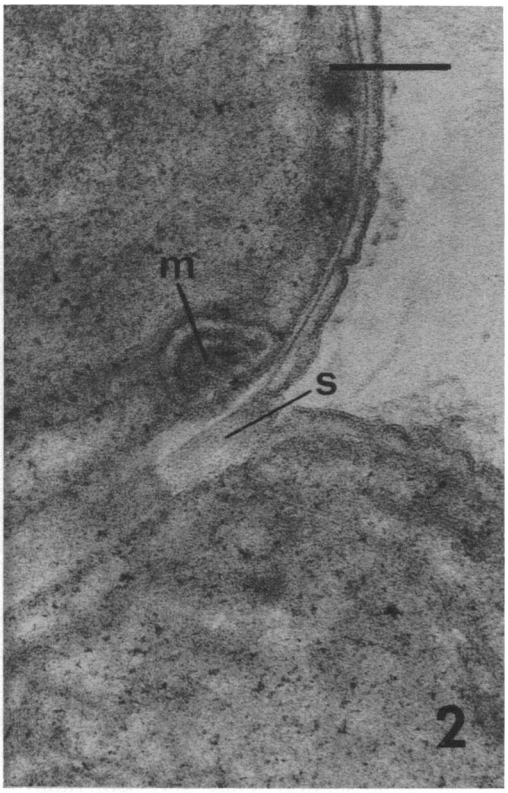
Penicillin G is known to inhibit synthesis of peptidoglycan in cell walls of bacteria, thereby leading to eventual cell lysis and death (7). At very low concentrations, however, the drug can inhibit initiation of cell division without blocking lateral elongation of the cell wall (10). Treatment of rod-shaped bacteria with such low concentrations of penicillin leads to the formation of long filaments in many species (4, 12). Ingram et al. (6) have reported a similar result in the unicellular cyanobacterium *Agmenellum quadruplicatum* and have described the ultrastructural characteristics of the resulting filaments in detail. To date, ultrastructural changes occurring in *A. quadruplicatum* before lysis at higher concentrations of penicillin G have not been reported. The object of this study was to determine whether any ultrastructural changes occur in *A. quadruplicatum* before lysis resulting from treatment with lethal levels of penicillin G. We report here the effects of penicillin treatment on mesosome-like structures that are present in this organism.

A. quadruplicatum strain PR-6 was isolated by Van Baalen (14). Broth cultures were grown at 39°C in medium A (13). Continuous agitation and CO_2 were provided by bubbling 3% (vol/vol) CO_2 in air through the cultures. Illumination was provided by four F24T12 CW/HO fluorescent lamps. Growth was measured turbidimetrically with a Bausch & Lomb Spectronic 20 colorimeter at 550 nm. After growth to an optical density of 0.8, cells were concentrated from the culture medium by centrifugation and suspended in 0.01 M tris(hydroxymethyl)aminomethane buffer (pH 7.2) containing 0, 15, or 75 U of penicillin G (1 U = 1 μg) per ml. The minimal inhibitory concentration of penicillin G for 10^7 cells per ml was found to be 0.0075 U/ml. These suspensions were incubated for 30 min as described above, after which samples were taken for electron microscopy.

Two fixation procedures were used in preparing cells for electron microscopy. For the Kellenberger fixation, cells were prefixed by adding 1% OsO_4 (in Kellenberger buffer [8]) to the cell suspensions until the final OsO_4 concentration reached 0.1%. The cells then were concentrated by centrifugation, washed in Kellenberger buffer, and embedded in 2% Noble agar (Difco Laboratories). The solidified agar was cut into small blocks which were postfixed overnight in 1% OsO_4 (in Kellenberger buffer) and then prestained for 3 h in 0.5% uranyl acetate (in Kellenberger buffer). For the Price fixation, concentrated cell pellets were suspended and fixed for 1 h at 4°C in a solution containing 0.5 ml of cold 4% glutaraldehyde, 1.0 ml of phosphate buffer (0.2 M, pH 7.2), and 0.5 ml of cold 4% OsO_4. They then were embedded in agar as above.

Samples from both fixations were dehydrated through a graded ethanol series and embedded in Spurr low-viscosity epoxy resin (11). Sections were cut on an LKB Ultratome III ultramicro-





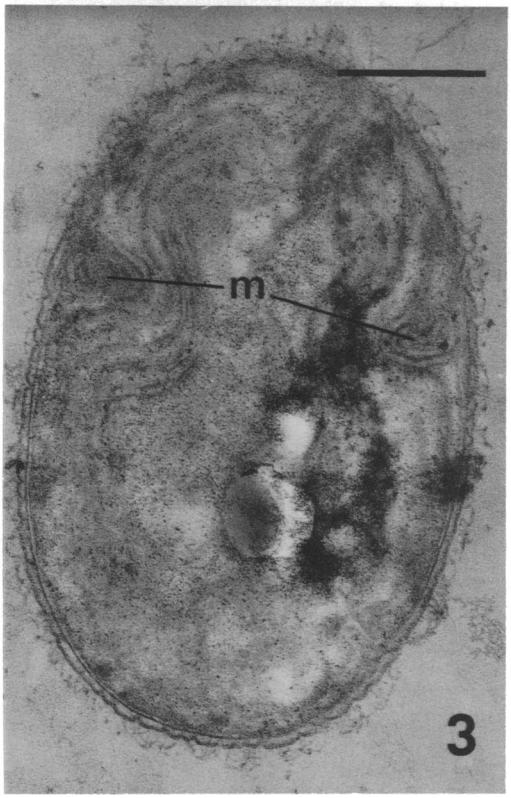


FIG. 1. *Electron micrograph of thin-sectioned A. quadruplicatum cell treated for 30 min with 15 U of penicillin G per ml. Typical mesosome-like structure (m) extends into the cell so as to disrupt the normal arrangement of thylakoid membranes (th). Kellenberger fixation. Bar = 0.2 μm.*

FIG. 2. *Electron micrograph of thin-sectioned A. quadruplicatum cell treated for 30 min with 75 U of penicillin G per ml. Mesosome-like structure (m) is associated with division septum (s). Kellenberger fixation. Bar = 0.2 μm.*

FIG. 3. *Electron micrograph of thin-sectioned A. quadruplicatum cell treated for 30 min with 75 U of penicillin G per ml. Mesosome-like structures (m) resembling ingrowing septum are located on opposite sides of the cell. Kellenberger fixation. Bar = 0.5 μm.*

tome and then poststained for 15 min with 0.5% uranyl acetate (in 50% methanol) and for 2 min with 0.4% lead citrate (9). Samples were examined in a JEOL JEM-100S transmission electron microscope at an accelerating potential of 80 kV. Only the results of the Kellenberger procedure are described here; similar results were obtained with the Price fixation.

Electron microscopy indicated that the number of mesosome-like structures present in *A. quadruplicatum* almost doubled as a result of treatment with 15 U of penicillin G per ml for 30 min. The average number of these structures in the untreated sample was 0.38/cell (standard deviation = 0.24), whereas an average number of 0.72/cell (standard deviation = 0.64) was observed in the penicillin-treated sample. The mesosome-like structures in both samples were of lamellar construction, and they usually appeared to be invaginations of the cytoplasmic membrane. They extended far enough into the cell to disrupt the normal arrangement of the thylakoids (Fig. 1). Thus, they resembled mesosomes reported previously for this (5) and other (2, 3) species of cyanobacteria. They were sometimes associated with the septa of dividing cells, but were also observed at other locations on the cell periphery. For both control and treated samples, the average diameter of the mesosome-like structures was 0.15 μm.

Treatment with 75 U of penicillin G per ml for 30 min resulted in a greater frequency of mesosome-like structures (1.06/cell; standard deviation = 0.39) than was seen for treatment with 15 U/ml. A *t*-test showed that the difference between control samples and those treated with 75 U/ml was significant at a 99.9% confidence limit. They were morphologically similar to those observed at the lower penicillin concentration, but their average diameter (0.18 μm) was slightly larger. Many of the structures in this sample were associated with septa in dividing cells (Fig. 2). Relatively large and complicated mesosome-like structures were seen on opposite side of some cells (Fig. 3). These structures resembled the division septa in other cells (Fig. 2), but they lacked peptidoglycan material.

The results of this study indicate that treatment of *A. quadruplicatum* with lethal doses of penicillin G leads to increases in the frequency and size of the mesosome-like membranous structures present in this organism. Since many of these structures were seen in association with the ingrowing septa of dividing cells, synthesis of membrane material during septum formation probably continued after peptidoglycan synthesis was blocked by penicillin. In this regard, some of the cells observed at 75 U/ml appeared to have carried out normal processes of septum formation, such as invagination of the thylakoids (1, 5), although the resulting septum-like structures lacked peptidoglycan material.

Increases in the frequency of occurrence of mesosome-like structures that were not associated with division septa also were observed as a result of penicillin treatment. It is not entirely clear why penicillin should cause the appearance of membranous structures in regions of the cell that are not involved in septum formation. One possible explanation is that such regions represent sites of lateral wall elongation at which peptidoglycan synthesis has been inhibited by penicillin. Alternately, they may represent initiation sites for the next round of cell division, as penicillin has been shown to block initiation of cell division in bacteria (10).

A direct correlation between penicillin G concentration and both the frequency and the size of mesosome-like structures was observed in this study. These results could indicate that penicillin treatment somehow stimulates synthesis of membranous structures in *A. quadruplicatum*, but such an effect would be difficult to explain in terms of the known actions of the drug. It is more likely that the results stem from a concentration-related effect on peptidoglycan synthesis. Both penicillin concentrations used in this study were sufficient to block all peptidoglycan synthesis and lead to eventual cell death (6). If complete inhibition of peptidoglycan synthesis had occurred more rapidly at the higher concentration, however, membrane synthesis without concomitant wall synthesis could have continued for a longer period of time than at the lower concentration. Consequently, the mesosome-like structures would have been somewhat larger.

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