Fine Structure of Spore Formation and Germination in *Micromonospora chalcea*

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INTRODUCTION

The genus *Micromonospora* contains filamentous bacteria which form a monopodially branched mycelium from one asexual spore. Spores are produced on this mycelium and, under appropriate conditions, these germinate and repeat the cycle.

The fine structure of the genus was studied by Luedemann & Casmer (1973) who related the mycelial and spore structure seen in thin sections to the silhouettes obtained by the electron microscopy of whole mounts. They presented electron micrographs of different stages of sporogenesis in *M. chalcea*, but all of them after the formation of the sporulation septum. The ultrastructure of sporogenesis was studied in *M. globosa* and *M. fusca* by Stevens (1975), and Sharples & Williams (1976) have described the ultrastructure of spore germination in *M. melanosporea*. However, we know of no previous studies of both sporogenesis and germination in the neotype strain *M. chalcea*.

METHODS

Culture conditions. *Micromonospora chalcea* ATCC 12452, neotype strain, (Luedemann, 1971) was inoculated by streaking on GAE solid medium (Hardisson & Manzanal, 1976) in Petri dishes and incubated at 28 °C. After 6 to 12 d, well-isolated colonies were selected for an ultrastructural study of sporulation.

Preparation of spore suspensions. Organisms were grown for 20 d at 28 °C in 250 ml flasks containing 75 ml GAE broth, and spore suspensions were prepared as previously described (Hardisson *et al.*, 1978).

Germination. Recently harvested spore suspensions were germinated as described previously (Hardisson *et al.*, 1978). Spores at different stages of germination (dormant, swollen and spores with germ tubes), as revealed by phase-contrast microscopy, were selected.

Electron microscopy. Samples for the study of sporulation and germination were treated as previously described (Hardisson & Manzanal, 1976; Hardisson *et al.*, 1978) except that they were stained with uranyl acetate for 30 min and poststained with lead citrate (Reynolds, 1963) for 4 min. Photographs were taken with a Philips EM-300 electron microscope operating at 80 kV.

RESULTS

Sporulation septum formation. The early stages of sporogenesis in *M. chalcea* were characterized by a lateral or apical swelling of a hypha and the formation of a sporulation septum (Fig. 1*a, b, c, d*). The sporulation septum had a three-layered structure similar to that of the vegetative cross-wall (Fig. 1*a*) from the onset of the process (Fig. 1*b*). Mesosomes were associated with the developing septum and the nuclear region (Fig. 1*a, b, c, d*). Warts were observed on the developing spore wall before completion of the sporulation septum (Fig. 1*b, c*) but more clearly after its completion (Fig. 1*d*). The cytoplasmic membrane seemed to follow the contours of the warts (Fig. 1*d*).

Spore maturation. After completion of the sporulation septum, a series of changes transformed the newly delimited swollen hyphal tip into a mature spore. There was a

Fig. 1. Sporogenesis in *Micromonospora chalcea*: Cw, vegetative cross-wall; N, nuclear material; M, mesosome; Wr, wart; SS, sporulation septum; Pm, plasma membrane; O, outer spore wall; C, medial spore wall; I, inner spore wall. Bar markers represent 200 nm.

(a) Initiation of sporogenesis by the swelling of the apical end of a hypha. A vegetative cross-wall showing a three-layered structure and nuclear material associated with a mesosome may be seen.

(b, c) Initial stages of sporulation septum formation. A mesosome is associated with the sporulation septum and with the nuclear material. Note the three-layered structure of the septum.

(d) A newly delimited spore showing a mesosome associated with the nuclear region and several warts on the original hyphal wall. The plasma membrane evaginates at the site of the warts.

(e) Spores in an early stage of maturation. The spore wall shows a three-layered structure, but all the layers are approximately equal in width.

(f) Mature spore after release showing an inner, thick and electron-dense layer, a middle less electron-dense layer and an outer, thin and electron-dense layer. No structures can be seen within the cytoplasm.

Fig. 2. Spore germination in *Micromonospora schalcea*: symbols as in Fig. 1. Bar markers represent 200 nm, except where indicated.

(a) Swollen spore showing the cytoplasmic membrane, nuclear material, abundant ribosomes and spore wall. The rigid outer spore wall layer has broken at several points.

(b) Germinating spore at an early stage of germ tube emergence. Note the continuity of the germ tube wall with the inner layer of the spore wall. Mesosomes may be seen as simple membranous pouches.

(c) Spore with a well developed germ tube having two nuclear regions, one of which has moved out to the germ tube and is associated with a mesosome.

(d, e) Enlarged detail of the spore wall in a dormant spore and in a swollen spore, respectively. Note the difference in width of the inner layers. (Bar markers represent 100 nm.)

centripetal deposition of wall material to form different layers (Fig. 1 *e, f*). The final mature spore had a wall comprising three zones of different electron densities: an outer electron-dense layer (about 7 to 8 nm), an intermediate less electron-dense layer (about 11 nm) and an inner electron-dense layer (about 25 to 26 nm). The thickness of the entire wall was about 44 nm. The diameter of the dormant spore was about 0.38 to 0.40 μm and its surface area was about 0.115 to 0.125 μm^2 . As maturation proceeded, the cytoplasmic structures of the spore, such as mesosomes, DNA and ribosomes, became less visible, probably because the fixatives could not penetrate the spore.

Germination. The first visible change in ultrastructure during germination coincided with the swelling stage (Fig. 2*a*). The spore diameter increased to between 0.54 and 0.56 μm and its surface area to about 0.23 to 0.25 μm^2 . Probably because of an increase in the permeability of the spore wall at this stage, the cytoplasmic structures were clearly seen. The thickness of the entire wall decreased to between 21 and 23 nm and, although the three-layered structure of the dormant spore wall was conserved, its appearance was quite different. The outer layer, probably because of its rigidity, was broken at numerous points as a consequence of the spore swelling, although it preserved the same electron density and thickness as observed in the dormant spore. The intermediate, less electron-dense layer also apparently remained unchanged, while the inner layer was noticeably thinner (9 nm) than in the dormant stage. It was this inner layer which extended and emerged through the outer layers to form the germ tube wall (Fig. 2*b*). The nuclear region in germinating spores had an irregular shape without defined boundaries (Fig. 2*b, c*). Mesosomes were not frequent during these early stages of germination; when present, they were simple membranous pouches (Fig. 2*b, c*), often in association with the nuclear material.

Changes in dimensions of spores and their walls during germination. During swelling, the spore perimeter increased and, simultaneously, the thickness of the wall changed from about 44 nm to about 21 to 23 nm. To estimate if stretching of the wall alone is sufficient to cover the enlarged surface area of the swollen spore, Gull & Trinci (1971) proposed the equation: $t_s = t_d \times (100/S)$, in which t_s is the thickness of the wall after swelling, t_d the thickness of the dormant spore wall and S is the surface area of the swollen spore, expressed as a percentage of the dormant spore surface area. Using this equation the estimated value for t_s (22 nm) was close to the value observed for the swollen spore wall indicating that synthesis of new wall material was not necessary during swelling.

DISCUSSION

Sporogenesis in *M. chalybeata* occurs in two stages, sporulation septum formation and spore maturation. The process is initiated by the swelling of the apical end of a hypha before its delimitation by the sporulation septum. Mesosomes are associated with the DNA and with the formation of the sporulation septum, as has been reported previously for other bacteria (Ryder, 1968; Greenawald & Whiteside, 1975). In *M. globosa* and *M. fusca*, Stevens (1975) did not find mesosomes directly associated with the septum until its completion. As Luedemann & Casmer (1973) pointed out, warts are formed on the spore wall before the deposition of the material of the new layers of the spore wall has begun. Perhaps the origin of the warts is linked to the swelling of the hyphal tip at the beginning of sporogenesis. The swelling of a hypha will need the participation of enzymes involved in wall lysis and synthesis. It is possible that warts are points of preferential wall attack and synthesis with less mechanical resistance. The sporulation septum has a typical three-layered structure similar to that of the vegetative cross-wall, as has been reported previously in *M. chalybeata* (Luedemann & Casmer, 1973) and in *M. fusca* and *M. globosa* (Stevens, 1975). The structure of the sporulation septum and its process of formation is similar to that of *Streptomyces* type I (Hardisson & Manzanal, 1976). However, the vegetative cross-walls in *Streptomyces* are formed by a septum of uniform electron density (Glauert & Hopwood, 1961; C. Hardisson & M. B. Manzanal, unpublished results), and the process of spore maturation is different. In *Streptomyces* (Manzanal & Hardisson, 1978) no layers are discernible in the spore wall, whereas in the mature spores of *M. chalybeata* there is a stratified deposition of materials with different electron density.

The ultrastructural changes that accompany the germination of *M. chalybeata* spores are similar to those found in *Streptomyces* (Hardisson *et al.*, 1978). In both cases it is the inner layer of the stratified spore wall which forms the wall of the germ tube. However, in *Strep-*

toomyces the spore wall only appears stratified after swelling. In both cases the nuclear material is irregular in shape without defined boundaries, an aspect that corresponds to the DNA in active transcription (Ryter & Chang, 1975). The structure of the germinated spore is different from that found in *M. melanospora* (Sharples & Williams, 1976) in which cross-walls develop soon after the emergence of the germ tube, and the cytoplasm of the spore shows abundant spherical electron-light areas; these were never observed in germinated spores of *M. chalicea*.

The swelling of the spore is apparently not accompanied by the synthesis of new wall material. The stretching of the inner layer in the dormant spore accounts for the increase in surface area. Similar results were found in *M. melanospora* (Sharples & Williams, 1976).

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