Electron Microscope Study of Whole Mounts and Thin Sections of *Micromonospora chalcea* ATCC 12452

GEORGE M. LUEDEMANN and CHARLES J. CASMER

Department of Antibiotics and Department of Physiology, Schering Corporation, Bloomfield, New Jersey 07003

The use of whole mounts in the electron microscope study of *Micromonospora chalcea* is a rapid and simple method for obtaining morphologic information about both the spore and sporophore. Spore anomalies were observed by this method, and their anatomical basis was confirmed by thin sections. Spore shape and surface ornamentation varied with the age of the spore and should be taken into consideration when characterizing and comparing the spores of various isolates. The spore of *M. chalcea* is not an endospore as found either in the bacilli or in the thermoactinomycetes; it lacks the multilaminar inner coat found in these thermoresistant spores. The spore of *M. chalcea* appears to contain an outer coat, or possibly coats, and a less electron-dense, thick inner coat or cortex. The spore of *M. chalcea* differs from the spore of streptomycetes in lacking a sheath around the hyphal wall. This sheath surrounding the hyphal wall in streptomycetes contains the spore ornamentation such as spines or hairs, but in *M. chalcea* spore ornamentation arises in the outer portion of the spore wall as wart-like protuberances. The spore of *M. chalcea* appears to develop its coats in a centripetal fashion similar to streptomycetes. Cross walls in *M. chalcea* are double and appear to develop in a manner similar to streptomycetes in which the hyphal wall appears to "peel apart," the inner portions being continuous with the cross walls. Mesosomes and nuclei found in thin sections of *M. chalcea* appear similar to those found in streptomycetes and other gram-positive bacteria. The electron microscope data obtained for *M. chalcea* ATCC 12452 in this study appear to be representative of the morphologic and anatomical features found in other species of *Micromonospora* that we have studied.

Spore silhouettes made by direct electron microscope observations of unfixed, unstained, or shadowed whole specimens have been used for characterization and comparison of the spores of species of actinomycete genera, in particular the genus *Streptomyces* (41, 44). These silhouettes, while useful for characterization and comparison of morphology, leave unanswered a number of anatomical questions as to what is internally responsible for the external morphology. These anatomical questions can be answered through the time-consuming method of fixation, embedding, thin sectioning, and staining. In this study an attempt was made to correlate morphology (silhouettes) with anatomy (thin sections), for this is important if the less time-consuming method of spore whole mounts is to be reliably used for characterization and comparison of strains of *Micromonospora*.

*Micromonospora chalcea* is the type species of the genus *Micromonospora* Ørskov (27), and ATCC 12452 has been designated as the neotype strain (29). Since the type species usually has an important bearing on our concept of the genus, we have studied in detail the structures and anomalies observed in this strain. Based upon this information and other workers' observations (M. W. Rancourt, Ph.D. thesis, Rutgers Univ., New Brunswick, N.J., 1963; references 1, 2, 20), an expanded description for the genus *Micromonospora* is recommended.

MATERIALS AND METHODS

Culture material. *M. chalcea* ATCC 12452 was grown on an agar medium consisting of 0.1% yeast extract (Difco), 0.1% glucose (Merck & Co., reagent...
grade), 0.1% soluble starch (Difco), 0.1% CaCO₃ (Merck & Co., reagent grade), and 1.5% agar (Difco) for 14 days at 24 to 26°C. A sparse sporulating growth is produced upon this medium. An open-ended glass melting point capillary tube was used to harvest a small amount of the surface growth of this culture by gently scraping the mycelium and spores from the agar surface. The dark sporulating material was placed in a small vial, and fixative was added preparatory to embedding and sectioning. In preparing culture material for whole mounts, sterile distilled water was added to the vials, and the sporulating material was rubbed against the side of the vial until a turbid suspension was obtained.

**Fixation and embedding.** Primary fixation proceeded at 4°C for 2 h in a solution made of 78 ml of 15% (vol/vol) gluteraldehyde in 0.1 M sodium cacodylate buffer (pH 7.1), to which was added, just prior to use, 12 ml of a 2% solution of osmium tetroxide in distilled water (wt/vol). Secondary fixation and procedures prior to dehydration were performed as described by Hubert et al. (17). Specimens were dehydrated in ethanol, cleared in propylene oxide, and, after brief immersion in propylene-EPON (1:1), embedded in Epon 812 (31). Thick sections (1 µm) of the block were stained with Paragon stain (33) and oriented under a light microscope. Thin sections were cut by using diamond knives on a Porter-Blum MT2 Ultratome and were stained with uranyl acetate and lead citrate (38). Sections were examined and photographed in a Philips EM 300 at an accelerating voltage of 60 kV.

**Whole-mount preparations.** A single drop of culture suspension was deposited on the surface of a Formvar-coated Athene grid (200 mesh). Excess water was drained from the grid, and the grid was immediately examined and photographed in a Philips EM 300 at 60 kV.

**RESULTS**

In unstained whole mount preparations (referred to as silhouettes as contrasted to thin sections), a monopodially branched mycelium constricted at intervals by cross walls may be observed (Fig. 1). On laterally situated side branches are produced single, electron-dense (black) spores. In whole mounts of these spores. Figures 17 and 18 show thin sections of the mycelium. The sporophore has been cut tangentially, and the young spore has been cut to reveal a truncate base with well developed cross wall and an irregular surface. A fibrous prokaryotic nucleus is present in both the spore and cells of the vegetative hypha. The spore wall is not differentiated; the wall appearing around the young spore is that of the vegetative hypha, which is of the same thickness and electron density as the walls of the mycelium.

Figure 5 shows a thin section of an older spore in which regions assignable to an inner wall or cortex, an outer wall, and a hyphal wall are present. As the cortical region of the spore develops, the fibrous appearing condition of the nucleus disappears. In Fig. 6 is shown a section of an elliptical spore with well developed cortex and spore wall. The old wall of the vegetative hypha within which the spore is produced appears to rupture in areas along the spore surface and is very thin at the base of the spore where separation occurs. The double wall at the base of the spore is characteristic of a cross wall. The concave condition of the wall of the septum of the sporophore appears to be brought about by the pressure of the expanding spore.

Figure 7 shows a cross section of a developing sporophore cluster in which progressive spore-wall development occurs with a concurrent disappearance of the fibrous type nucleus in the older spores. Peripheral membrane activity often appears to increase with the thickening of the cortical layer. The occurrence of more than one spore at the apex of a sporophore is shown in Fig. 8-10. The apparent dichotomy has been termed a pseudodichotomy (28). In Fig. 11-13, sections of spore pairs differing in spore age, based upon cortical and nuclear development stages, lend credence to the observation that what appears to be a dichotomy (spores produced simultaneously) usually represents an older spore subtended by a younger spore (spores produced sequentially). In Fig. 9 the less electron-dense sporophore to the right in the spore pair is believed to be the younger spore in which little spore wall material has been laid down, thus accounting for its low electron density comparable to that of the vegetative mycelium.

Figure 14 presents a whole-mount silhouette of two septate mycelial filaments with clearly defined sessile spores. The small bud on the hypha on the right may be a spore or branch initial. Figures 15 and 16 are higher magnifications of the spores appearing in the middle of Fig. 14. Small warts appear along the surface of these spores. Figures 17 and 18 show thin
FIG. 1. Whole-mount silhouette (WMS) illustrating Ørskov's original concept of the genus Micromonospora in respect to spores produced singly on lateral side branches. The following abbreviations are used in the figures: B, branch or spore bud; Bcw, bifurcate cross wall; C, cortex; Cw, cross wall or septum; H, hypha; Hw, hyphal or vegetative cell wall; Hwr, hyphal wall remnant; M, mesosome; N, nucleus; Osc, outer spore coat or coats; Pm, plasma membrane; Sc, spore coat; Sp, spore; Sph, sporophore; Ss, sessile spore; Wb, wall blister; Wr, wart; V, storage vacuole (12, 18, 32). The scale marker in WMS is equal to 1 μm except in Fig. 2, 3, 15, and 16, where the marker equals 0.5 μm. The marker in all thin sections is equal to 0.5 μm.

FIG. 2. WMS of a young, relatively smooth walled, truncate spore. The truncate end represents the plane of attachment to the sporophore.

FIG. 3. WMS of a mature, moderately warty-walled spore.

FIG. 4. Longitudinal section of hypha, lateral sporophore, and very young truncate spore. No spore-wall material appears at this early stage of development.

FIG. 5. Longitudinal section of hypha, lateral sporophore, and spore with two fairly well developed spore coats.

FIG. 6. Longitudinal section of older spore with thick cortex, outer spore coat, and hyphal wall showing signs of rupture particularly at the thinning area adjacent to the sporophore. It is conceivable that some spores at this stage may be forcibly discharged as evidenced by the concavity of the cross wall on the sporophore side and convexity of the fragment of cross wall on the spore side.
sections of sessile spores with well developed cortices which have been cut transversely to the axis of the hyphae. Figure 19 shows a longitudinal section of a hypha and sessile spore with a well developed cortex and a younger, terminally produced spore to the left. In Fig. 20 is shown a longitudinal section of a septate hypha and a spore, borne on a very short sporophore, the condition of which resembles that of the spore on the left side of Fig. 14.

In whole mounts of *M. chalea* prepared for spore silhouettes, longitudinally paired spores occur. In Fig. 21 and 22 are longitudinal pairs of spores attached to their sporophores, and in Fig. 23 is a sessile longitudinal spore pair. Triple longitudinal spores are found in Fig. 24 adjacent to a single sessile spore. Extensive searching of thin sections produced several longitudinal spore pairs, one of which is shown in Fig. 25 attached to its sporophore. In Fig. 26 another spore pair occurs; the spores have well developed cortices and a double cross wall separating them.

Septa appear with more or less regularity in the mycelium. The mycelial diameter may vary between 0.4 and 0.8 μm. In Fig. 27 is a hypha of the larger diameter which is profusely septate. Figure 28 is a segment of mycelium in which the terminal cell on the left is blunt with rounded corners suggestive of what might be envisioned if rupture had occurred in the mycelium in Fig. 27 at the septa shown below the abbreviation Hwr. From this terminal cell a spore has been produced, and a rim of cortical material may be found. The other end of this hypha bears a cell comparable in size to a spore but lacking cortical and spore wall development. A transverse septum has formed in this cell, and a longitudinal septum is being formed. This appears similar to a chlamydospore lacking the inner and outer wall development characteristic of a normal spore. Figure 29 shows a
FIG. 8. WMS of spore pair produced on a short lateral sporophore.

FIG. 9. WMS of spore pair in which one member of the apparent dichotomous pair is of the same electron density as the mycelium. A sessile spore is also present.

FIG. 10. WMS of spore pair produced on a long sporophore appearing in silhouette as an apparently perfect dichotomy. There would be little value in attempting to use the length of the sporophore in classifying this strain (4, 42).

FIG. 11. Thin section of a spore pair thought to be similar to what a section of the spore pair in Fig. 8 would appear like. The older spore is believed to be on the left.

FIG. 12. Thin section of a spore pair illustrating how internal anatomy of a spore changes with spore-wall development. We believe the spore on the right in which spore-wall material has not yet been deposited would be comparable in electron density to that of the spore on the right in Fig. 9; i.e., it would have the same low electron density as the hyphal wall. Sites just beneath the hyphal wall of this spore causing bulges in the wall appear to be indicative of areas of wart formation, as is also indicated in Fig. 4.

FIG. 13. Thin section of two adjacent spores believed to be of slightly different age based upon cortical development. Origin of surface warts appears to be in the outer wall layer or fibrous layer as described by Rancourt (Ph.D. thesis).
FIG. 14. WMS of septate mycelium bearing sessile spores.
FIG. 15 and 16. Higher magnification of central spores in Fig. 14 illustrating surface, shape, and truncate basal attachment.
FIG. 17 and 18. Transverse thin sections of hyphae and sessile spores.
FIG. 19. Longitudinal thin section of hypha and sessile and terminal spores.

triseptate section of a cell (chlamydospore) which might be the result of such a division as seen in Fig. 28. Figure 30 is of a longitudinal thin section of a similar multiseptate hypha or chlamydospore.

DISCUSSION

Modification of generic description. To the original observations made by Ørskov (36) of Streptothrix chalcea Foulerton (M. chalcea (Foulerton; Ørskov), which forms the basis for the generic characterization of the genus Micromonospora, may be added the following confirmations, corrections, and additions made possible by electron microscopy. Ørskov observed that the spores of this organism were constantly situated singly at the tip of usually short side branches (Fig. 1). Further, he noted that the mycelium never divides by septation and that septa do not arise at the base of the spores (Fig. 4). This statement of a characteristic, aseptate mycelium must now be corrected based upon the evidence of thin sections (1, 2, 20). Septa are produced and are characteristically double walled, as is also the case with streptomycetes (M. W. Rancourt, Ph.D. thesis, Rutgers Univ., New Brunswick, N.J., 1963; references 12, 35, 37, 45). Ørskov observed that the sporophore often is seen to grow gradually thinner at its extremity near the spore so that, supposedly by a sort of constriction process, the spore is parted from the sporophore (Fig. 6). We may add that the spore is separated from the sporophore by a cross
wall and appears to be liberated by the rupture of the stretched and thinned hyphal wall in the vicinity of this cross wall. In respect to spore liberation, the Micromonospora spore is like that of a streptomycete: it is dependent upon the rupture of the enclosing parent hyphal wall. The use of the term aleuriospore by Cross (6) for the Micromonospora spore is not unreasonable; however, anatomically it is a specialized, broad-based type of aleuriospore (aleurium, see reference 40).

In addition to Ørskov's observation of single spores produced on terminal or lateral branches, we may add that sessile spores are produced by a direct budding out of a spore from the hypha without the intervention of a sporophore. Such spores were pictured by Jensen (19) and others (23, 29, 30).

The occurrence of more than one spore on a lateral or terminal sporophore was pictured by Jensen (19), Kriss (22), and others (26, 30). Baldacci and Locci (3) used the degree of spore clustering in describing the subspecies Micromonospora melanosporea subsp. corymbica. Krasil'nikov and Agre (21) interpreted this clustering of spores in Micromonospora fusca as a true dichotomy, but this interpretation of the juxtaposition of spores as a dichotomy has been questioned (7, 24, 28). Figures 7, 11, and 12 of thin sections of such dichotomies and clusterings indicate that the spores are not of equal development and therefore are not of equal age and represent sequential spore formation, not simultaneous spore formation as would be expected in a true dichotomy. To Ørskov's generic description we would add that single spores produced in clusters of two or more spores most often originate sequentially rather than simultaneously, and this represents a condition of terminally congested spores on a sporophore, not a true dichotomy.

Perhaps the major addition to Ørskov's original description that electron microscopy can contribute is conclusive evidence of what might be called anomalous spores. Spores of M. chalcea (and of other species of Micromonospora that we have studied) occasionally are not produced singly but occur in longitudinal pairs or even small chains. Rancourt (Ph.D. thesis, Rutgers Univ., New Brunswick, N.J., 1963) first mentioned this, but did not show convincing evidence, i.e., two or more spores attached longitudinally to a sporophore. Longitudinal pairs of spores were pictured in whole mounts of Micromonospora brunea (23), but no comment was made by the authors on this condition. Spores occurring in longitudinal pairs and in small chains had been seen by the senior author of this paper in 1963 in a whole mount of spores of Micromonospora echinospora but were dismissed then as merely being "sticky" spores that clung together in the aqueous preparation when dried down on the electron microscope grid. Lechevalier and Pramer (26) pointed out longitudinal pairs of spores in a phase-contrast photomicrograph of

FIG. 20. Longitudinal thin section of hypha and spore borne on exceedingly short sporophore similar to spore at the left in Fig. 14. The diameter of the multisepate hypha is approximately twice the diameter of the sparsely septate hypha below it.
FIG. 21. WMS of a normal and an anomalous longitudinal spore pair.
FIG. 22. WMS of lateral sporophore and an anomalous longitudinal spore pair.
FIG. 23. WMS of an anomalous sessile longitudinal spore pair.
FIG. 24. WMS of an anomalous longitudinal spore triplet and a sessile spore. The low electron density of the spores is believed to be indicative of the absence or very early development of spore coats.
FIG. 25. Longitudinal thin section of anomalous pair of young spores attached to a sporophore. A spore coat has not been laid down.
FIG. 26. Thin section of anomalous longitudinal spore pair with partially developed cortex and outer coat. Note plane of cross walls.
**FIG. 27.** Thin section of a profusely septate hypha. Each cell appears capable of functioning as a unit of vegetative reproduction similar to a fungal arthrospore.

**FIG. 28.** Thin section of a multiseptate hypha and terminal chlamydospore.

**FIG. 29.** Thin section of a multiseptate structure similar to a cross section of a chlamydospore.

**FIG. 30.** Thin section of a multiseptate structure similar to a longitudinal section of a chlamydospore.

*M. chalcea* (Fig. 31). In whole-mount preparations made in this study to determine spore silhouettes, we discovered a number of longitudinal pairs of spores attached to their sporophores and a chain of three young spores (Fig. 21-24). A search of thin sections led to the discovery of longitudinal pairs of spores which left no doubt that they do occur although rarely enough to be considered anomalous in occurrence. Ørskov's original description that spores occur only singly at the end of sporophores in the genus *Micromonospora*...
would be modified to include the statement that infrequently spores may occur in longitudinal pairs and more infrequently as multiple longitudinal spores. We believe that these longitudinal pairs and multiple spores occur through a misfunction of normal septal division. Henssen (14) reported similar longitudinal spores in the genus Thermomonospora.

The last addition we would make to the description for the genus Micromonospora refers to the closely spaced, multiple septate, vegetative hyphal cells which appear similar to chlamyduospore forms found in fungi. The statement would include that often bizarre shaped and septate cells are found among certain enlarged vegetative hyphae and resemble the chlamyduospores found in filamentous fungi or involution forms in bacteria (18).

Spore formation. A full account of the anatomy of sporulation cannot be made in this paper; however, some comments based upon the electron micrographs may contribute to such an anatomical study in the future. Kenner, Hohl, and Baker (20) observed that spore formation in Micromonospora species was initiated by the formation of a septum which isolated the apical end of a hypha and within which a spore was produced. Fitz-James and Young (11) considered that sporulation may be thought of as a process of cellular division and orderly development of two main layers, a cortex and coats. Their hypothesis was based upon unicellular, endospore-forming bacteria, but it seems also worth pondering its significance in the case of Micromonospora spore formation. In thin sections it is seen that the double septum is found at the base of all spores in which the sporophore is also present. The spore of M. chalcea does not, however, form in a manner similar to that of endospore formation in unicellular bacteria or Thermoactinomyces species (7, 9, 11, 25). The M. chalcea spore is not a heat-resistant spore and does not contain an inner multilaminar coat. The M. chalcea spore bears a resemblance to the spore of the streptomycetes in that spore coats appear to be laid down centripetally. The M. chalcea spore appears to differ from the streptomycete spore in that its formation is an orderly deposition of an outer and an inner spore coat and not merely a thickening of the hyphal wall in which it is produced (10, 12). The hyphal wall is distinct from the spore coat and may be partially sloughed off in mature spores in which cortical development is complete. The spores of M. chalcea are not formed within a sheath which surrounds the hyphal wall as in streptomycetes (12, 13, 37, 44), and the spore surface warts or blunt spines are formed in the outer coat (or coats), thus differing from the surface ornamentation found in the sheath of some streptomycete spores (37, 44). The spore surface ornamentation of M. chalcea ATCC 12452 was classified as smooth by Maximova and Sveshnikova (34) from spore silhouettes obtained of this strain. Young spores may appear smooth walled (Fig. 2), whereas older spores may appear with a multitude of small
warts (Fig. 3). Blunt spines appear on the surface of some species of *Micromonospora* (27). Although they do not originate as the spines which appear in the sheath of some streptomycetes, they are sharp, acute projections which are difficult to call warts although they appear to arise in the same outer coat layer that the less acute or warty protuberances arise (M. W. Rancourt, Ph.D. thesis, Rutgers Univ., New Brunswick, N.J., 1963; reference 34).

The *Micromonospora* spore may be hypothesized to develop as the initial result of the isolation of a single apical cell by the formation of a double cross wall. This initial isolation step is followed by a centripetal movement of the plasma membrane as the membrane deposits spore wall material between it and the hyphal wall (39). An outer wall is laid down which is less electron dense (stains less intensely) than the hyphal wall and within which may be deposited excess spore wall material which accumulates as warty protuberances beneath the hyphal wall. As the plasma membrane moves centripetally, due to the deposition of wall material centrifugally, a second wall layer may be detected that is less electron dense and is often honeycombed by the plasma membrane in a characteristic fashion. As the inner coat develops, it appears that the outer spore wall is compressed against the hyphal wall and that the hyphal wall is stretched to the point of rupture at a number of points on the spore surface and at the junction with the sporophore. This expansion in spore diameter appears to result in spore liberation through rupture of the hyphal wall of the sporophore and often results in a roughened appearance of the spore surface, as is seen in thin section (Fig. 6). Whether the thick inner coat surrounding the spore core is termed a cortex (1, 2, 25) or simply an inner wall will be a point for spore anatomists to resolve.

**Spore shape.** With respect to information on spore shape and ornamentation derived from whole-mount silhouettes, caution must be used in their use for characterizing and comparing the spores of different isolates. The shape of an immature spore (Fig. 2) may differ from that of a mature spore (Fig. 3; reference 4), as may also the surface ornamentation. Maximova and Sveshnikova (34) interpreted spore silhouettes of *M. chalcea* ATCC 12452 as smooth; however some modification of their observation must be made to include a spore of *M. chalcea* ATCC 12452 as seen in Fig. 3. Spore-wall ornamentation in strains and species of *Micromonospora* requires further comparative study if it is to be a useful diagnostic characteristic. Wall ornamentation in the form of wart-like protuberances may appear very early in spore development as bulges in the hyphal wall before spore coat deposition is apparent (Fig. 4, 12). The significance of these early appearing bulges in the hyphal wall of the spore, which appear to be the primordial areas for wart formation and perhaps wall formation, is of interest in attempting to understand spore wall and ornamentation development. The information obtained from whole-mount silhouette studies of *M. chalcea* ATCC 14252 indicates the value, simplicity, and speed that this method offers, particularly when critical points of morphologic information are substantiated through thin-section comparisons.

**Cross walls.** Cross-wall formation in unicellular bacteria has been described by Higgins and Shockman (16), Higgins and Daneo-Moore (15), and in filamentous bacteria by Glauert and Hopwood (12), Rancourt and Lechevalier (37), Bradley and Ritzi (5), and Wildermuth and Hopwood (45). Cross walls in *Micromonospora* were observed by Arai et al. (1), Arai and Kuroda (2), and Kenner et al. (20), but the process of cross-wall formation was not discussed. Glauert and Hopwood (12), Rancourt (Ph.D. thesis, Rutgers Univ., New Brunswick, N.J., 1963), and Bradley and Ritzi (5) described cross-wall formation in streptomycetes in terms of a hyphal wall which appeared to be composed of two layers; the inner layer of this wall grew inward to form the cross wall. Wildermuth and Hopwood (45) have challenged this double-layer wall concept believing that their evidence indicates that the hyphal wall consists of one layer, not two, and they diagrammatically propose a cross-wall model differing from the earlier model of Glauert and Hopwood.

We have seen in the septate mycelium of *M. chalcea* (Fig. 27-30) evidence which is in agreement with that presented by others for cross walls formed in streptomycetes (5, 37). The wall appears to "peel apart" (Fig. 27; references 5, 16). We also find the bi- and trifurcate septa mentioned by Moore and Chapman (35) for a streptomycete and by Arai et al. (1) for a micromonospora. We conceive the cross walls to originate as a unilateral blister in the hyphal wall which proceeds to develop much like an iris diaphragm cutting across the protoplasm. Mesosomes appear to be involved (16, 43). It is believed that this unilateral blister hypothesis is more consistent with our observations than either the double-wall hypothesis of Glauert and Hopwood (12) or the hypothesis of Wildermuth and Hopwood (45). It is somewhat similar to the model proposed by
Rancourt and Lechevalier (37). The blister hypothesis may also be helpful in explaining the bi- and trifurcations observed frequently at the junction of the cross wall and hyphal wall.

The occurrence and spacing of cross walls in the hyphae of *Micromonospora chalcea* ATCC 12452 is of interest, and a trend in septal occurrence has been noted. The smaller-diameter hyphae (approximately 0.4 μm) are sparsely septate (Fig. 20). The larger-diameter hyphae (approximately 0.8 μm) are often abundantly septate. Cross-wall frequency appears to be a direct function of mycelial diameter. The opinion is offered that septation may be a function of the nuclear cytoplasmic ratio. The amount of nuclear material per cell is probably relatively constant, and as the diameter of a cell increases, the balance between nuclear material and cytoplasm is normally regulated by cross wall formation.

Mesosomes appear frequently in the mycelium and spores of *M. chalcea* as they also do in the spores and mycelium of other actinomycetes (25, 43). Kenner et al. (20) did not find these lamellar organelles in the *Micromonospora* species they studied, which may have been due to unfavorable conditions in the fixation and staining procedure they used for resolving these organelles.

A typical bacterial nucleus occurs in the cells and young spores of *M. chalcea*, as was also noted by Kenner et al. (20) and suspected by Jensen in 1930 (19).

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REPRINT REQUESTS

Address requests for reprints to: Dr. George M. Luedemann, 46 Lincoln St., Glen Ridge, N.J. 07028.

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