Electron Microscopic Observation of the Sporangial Structure of an Actinomycete, *Microellobosporia flavea*

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SUMMARY

Ultrathin sections of sporangia of the actinomycete *Microellobosporia flavea* were examined with an electron microscope. The sporangial wall is a thin, wrinkled membrane which seems to be an extension of the outer layer of the cell wall of the sporangiophore. A substance, probably a liquid, is located between the sporangial wall and the sporangiospores. The spores are ovoid and consist of a laminated wall in which two layers can be differentiated. The wall is 30-40 m μ thick but thickens to 90-120 m μ at the point of contact between two spores. Inside the spores one can differentiate a finely granular nucleus, large vacuoles which are probably filled with a fatty substance, and a coarsely granular cytoplasm. The cytology of the sporangiospores of *M. flavea* is similar to that of the conidia of *Waksmania rosea*.

INTRODUCTION

More than a decade ago the isolation of actinomycetes which formed motile sporangiospores was reported (Couch, 1949). Later Couch proposed to call such organisms by the generic name *Actinoplanes* (Couch, 1950) and he described, under the name *Streptosporangium* (Couch, 1955), actinomycetes which formed non-motile sporangiospores. He also suggested that the family Actinoplanaceae be created to group all sporangia-bearing actinomycetes (Couch 1955*a*). Strains of both *Actinoplanes* and *Streptosporangium* form globose to spherical sporangia containing numerous spores which are randomly distributed at maturity. Recently, organisms which form non-motile sporangiospores arranged in a short single row have been described under the new generic name, *Microellobosporia* (Cross, Lechevalier & Lechevalier, 1963). Two species of this new genus of *Actinoplanaceae*, differing from each other mainly in pigmentation, were described: *M. cinerea*, the type species; and *M. flavea*. Cross and his co-workers showed the presence of the sporangial wall by light microscopy and by electron microscopy, and they followed with light microscopy the formation of the spores within the sporangia. They noted that even though all the spores within a sporangium were of the same age, they were not necessarily of the same size. In the present paper electron microscopy is used to probe the structure of sporangia and sporangiospores of *M. flavea*. Sporangia are borne both on the substrate and on the aerial mycelium, but only the structure of the more accessible aerially-borne sporangia was investigated. These are club-shaped structures borne on the swollen tips of sporangiophores and usually contain from 2 to 5 spores. They range in length from 2 to 9 μ and in diam. from 1.5 to 8.6 μ .

METHODS

Organism. *Microellobosporia flavea*, No. 8858 (Culture collection of the Institute of Microbiology of Rutgers).

Growth and preliminary treatment of the organism. Potato + carrot agar medium (Segretain, Drouhet & Mariat, 1958) was poured into Petri dishes. The surface of the solid medium was covered with a film of cellophan (Chabbert & Patte, 1960) and inoculated with a cell suspension which was spread over the surface of the cellophan. The plates were incubated at 28° until mature sporangia were abundant (about 10 days). The cellophan membranes were removed from the medium and the growth that they carried was scraped off with an inoculating needle. The material thus collected was suspended in acetate-veronal (sodium barbital) buffer (Kellenberger, Ryter & Séchaud, 1958) and was sedimented by centrifugation.

Fixation. The procedure of Kellenberger *et al.* (1958) was followed with minor modifications. The fixative was a 1 % (w/v) solution of osmium tetroxide in acetate-veronal buffer (pH 5.9) to which traces of calcium chloride were added just before use. The previously sedimented organisms were suspended in this fixative and left there overnight. After proper centrifugation and washing, the fixed organisms were embedded in 2 % (w/v) agar which was cut in small (1–2 mm.³) blocks. These were suspended in 0.5 % (w/v) aqueous uranium acetate for 1 hr.

Embedding. After fixation, the specimens were dehydrated with ethanol. After having been passed twice through propylene oxide, they were embedded in Epon 812 (Shell Chemical Co., San Francisco) according to the method of Luft (1961) by using 3 volumes of his mixture A for 7 volumes of his mixture B.

Thin sectioning. After proper paring, the resin blocks were sectioned either with a Porter-Blum microtome (Ivan Sorwall, Inc., Norwalk, Connecticut, U.S.A.) equipped with glass knives or with an LKB Ultratome microtome (L.K.B. Instruments, Inc., 4800 Rugby Avenue, Washington 14, D.C., U.S.A.) equipped with a diamond knife. The sections were floated on 10 % (v/v) acetone in water and were picked up on collodion coated grids.

Electron microscopy. The sections were examined with an RCA-3D electron microscope (aperture, 25 μ) operated at 50 Kv., and photographed on Eastman Kodak Lantern Slides at an instrumental magnification of 8000.

RESULTS AND DISCUSSION

Not a single section was obtained which passed through the whole sporangium and sporangiophore. This is not surprising when one considers the large size of this whole structure. At best, some sections were obtained which passed through the apex of a sporangiophore to which two spores were still attached (Pl. 1, fig. 1), or sections were obtained through three spores but not the sporophore (Pl. 1, fig. 2).

The sporangial wall appears as a thin, loosely-wrinkled membrane which looks like an extension of the outer covering of the cell wall of the sporangiophore (Pl. 1, figs. 1 and 3). Inside the sporangium, between the spores and the sporangial wall, is located an electron light, reticulated to granular substance. It is difficult to measure accurately the thickness of the sporangial wall, since one is never sure that the wall has not been cut obliquely, but this thickness was estimated at 5–6 m μ .

Sections of spores, cut perpendicularly to the long axis of the sporangium, are round. Cutting the spores through the long axis of the sporangium revealed an ovoid shape. The walls of the spores are laminated and two main layers of different density can be seen in most sections, the outer layer being the darker one. The spore walls are 30–40 $m\mu$ at the equator and thicken into pads at the point of contact between two spores. These pads (90–120 $m\mu$) are mainly due to the thickening of the outer layer. The spore walls exhibit another anatomical feature which is always located in the area where the thickening of the interspore pads starts. It is reminiscent of a plasmodesm and might represent a point of cytoplasmic contact between two adjacent immature spores before they are separated by the obesity of maturity, or it might indicate a point of contact, at some stage of maturation, between the cytoplasm of the spores and the sporangial membrane. Such structures can be seen on Pl. 1, figs. 1 and 2; in fig. 1 two arrows point to a pair of such structures located on adjacent spores. In addition, the interspore pads and more strikingly the spore-wall pad which rests on the sporangiophore, exhibit two electron light areas which look like an extension of the inner spore wall layer. On Pl. 1, figs. 1 and 3, these areas appear as two oblique lines converging toward the inside of the spore.

Within the spores three main cytological structures can be noted. (1) A large ovoid, more or less centrally located mass of a very finely granular substance; by its very location, one would be tempted to call it a nucleus. Its structure is different from that previously observed in 'nuclei' of spores of *Streptomyces* which had been fixed, stained and embedded by the same method used here for *Microellobosporia flavea*. (2) Numerous electron-light vacuoles. (3) A coarsely granular material, darker than the 'nucleus', which fills up whatever space is left, and which is probably the cytoplasm. Since the spores of *M. flavea* were easily stained with Sudan Black it is a fair assumption that the large vacuoles are formed by the deposition of a fatty storage material. An attempt to identify this compound as poly- β -hydroxybutyric acid failed (Slepecky & Law, 1960).

Macroscopically, the growth of strains of *Microellobosporia* resembles that of *Streptomyces*; microscopically, at least at first glance, it is reminiscent of *Waksmania* (Lechevalier & Lechevalier, 1957). It is interesting that sections of spores of *M. flavea* are very similar to sections of spores of *W. rosea* (Pl. 1, fig. 4) and somewhat different from what we would consider a typical section of a spore of a *Streptomyces* (Pl. 1, fig. 5). By comparing these pictures of organisms which have all been treated in the same manner one will note the greater abundance of vacuoles in *Microellobosporia* and *Waksmania* than in *Streptomyces*, and the rather light unevenly-dense nuclear material of the *Streptomyces* which contrasts with the evenly-granular structures of the other two organisms. Study of additional strains of these and related organisms may permit some generalizations with phylogenetic implications. Along this line of thought, we are tempted to compare the sporangial wall in *Microellobosporia* with the outer layer of the sporogenic hyphae of *Streptomyces*, which breaks up into spore-sized segments, and which finally enrobes each mature spore (Glauert & Hopwood, 1961). *Microellobosporia* could then be considered as short chained *Streptomyces* in which the outer layer of the sporogenic hyphae does not break up and does not cover each spore. Rather, this outer layer remains continuous around the whole chain of spores, and some fluid is probably

present between the sporangial wall and the spores. *Waksmania* may well be a link between *Microellobosporia* and *Streptomyces* since its spores are non-sporangial like the *Streptomyces*, and its cytology is strikingly similar to *Microellobosporia*.

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EXPLANATION OF PLATE 1

Electron microphotographs of thin sections of spores of Actinomycetes.

- Fig. 1. Section of sporangiophore and sporangium of *Microellobosporia flavea* 3858. $\times 22,200$.
- Fig. 2. Section of sporangium of *M. flavea* 3858. $\times 20,000$.
- Fig. 3. Section of sporangiophore and sporangium of *M. flavea* 3858. $\times 24,000$.
- Fig. 4. Section of spore of *Waksmania rosea* 3748. $\times 29,600$.
- Fig. 5. Section of spores of *Streptomyces violaceus* 829. $\times 21,000$.