Fine Structure of the Globose Bodies of *Dactylosporangium thailandense* (Actinomycetales)

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The genus *Dactylosporangium* (Thiemann, Pagani, & Beretta, 1967) contains actinomycetes which form elongated sporangia, each containing a single row of spores. This feature, together with the presence of *meso* 2,6-diaminopimelic acid in the cell walls (Lechevalier & Lechevalier, 1970), places it in the family Actinoplanaceae. In addition to sporangia, globose bodies (1.7 to 2.8 μm diameter) are formed on the substrate hyphae. These do not release spores and attempts to germinate them failed (Thiemann *et al.* 1967). They were proposed as an additional morphological characteristic of this genus (Thiemann, 1970*a*). This paper reports a study of the fine structure of globose bodies in *Dactylosporangium thailandense* (= *thailandensis*, Thiemann, 1970*b*) and considers their possible significance.

METHODS

Cultures of *Dactylosporangium thailandense* (ATCC23409) were grown on oatmeal agar or colloidal-chitin medium (Lingappa & Lockwood, 1961) at 25 °C for 4 weeks. Periodic examination of the cultures by light microscopy indicated whether globose bodies or sporangia were present. Small blocks of medium with growth were removed from suitable cultures and prepared for transmission and scanning electron microscopy.

For the former, blocks were fixed in buffered 1 % (w/v) osmium tetroxide for 16 h at room temperature, washed in 0.5 % (w/v) uranyl acetate for 3 h, dehydrated with ethanol, embedded in an epoxy resin (Spurr, 1969), and ultra-thin sections were prepared. These were examined with an EM6B electron microscope (AEI Scientific Apparatus Ltd) operated at 60 kV.

For scanning electron microscopy, blocks were quenched in iso-pentane cooled to –150 °C, freeze-dried in a Pearse-Edwards tissue dryer, coated under vacuum with a thin film of gold–palladium, and examined with a Stereoscan electron microscope (Cambridge Scientific Instruments Ltd) operated at 20 kV.

RESULTS AND DISCUSSION

Formation of sporangia was erratic, particularly on oatmeal agar. In cultures producing few sporangia, globose bodies were frequent, while few were formed when sporangia were abundant. Numbers of globose bodies also increased as cultures aged.

Scanning electron microscopy of cultures producing many globose bodies showed that many hyphae were lysed. In addition to large smooth-walled globose bodies, many smaller swellings occurred, and the hyphae were surrounded by much particulate debris (Fig. 1*a, b*). As a result of hyphal lysis, globose bodies were eventually freed from their parent hypha, sometimes retaining their hyphal stalk (Fig. 1*b*). Free globose bodies were surrounded by particulate debris which presumably arose from breakdown of the hyphae. Less regularly

Fig. 1. (a) Large globose body on hypha with smaller globose structures, lysing hypha and particulate debris (scanning electron micrograph). (b) Detached globose body with hyphal stalk (scanning electron micrograph). (c) Development of cross wall-delimiting globose body. (d) Globose body with diffuse light areas, defined light areas and possible phage particles. (e) Globose body attached to lysed hypha, and containing a crystalline body. (f) Crystalline body.

shaped, enlarged structures were also occasionally observed. Both these and the globose bodies appeared to be more resistant to lysis than the hyphae which produced them.

Ultra-thin sections of globose bodies showed that they were bounded by an homogeneous wall which was somewhat thicker (20 to 30 nm) than that of the hyphae (12 nm). They were separated from the lysing parent hypha by ingrowth of a cross wall of the kind designated as type II by Williams, Sharples & Bradshaw (1973) (Fig. 1c). The cytoplasm usually contained diffuse electron-light areas and smaller, clearly-defined light areas (Fig. 1d). The latter was bounded by a single electron-dense line and varied in diameter from 70 to 180 nm. In larger globose bodies, the cytoplasm was often more dispersed, a phenomenon also noted by Lechevalier & Lechevalier (1969) in the larger vesicles of the actinomycete *Intrasporangium calvum*. Dispersion of the cytoplasm may account for the 'empty' globose bodies observed in the light microscope by Thiemann *et al.* (1967). The most unusual feature of the cytoplasm was the presence of darkly stained lamellate bodies (Fig. 1e, f). The width of these was from 70 to 180 nm and their length was very variable, but they were usually cigar-shaped. They consisted of alternating electron-dense and electron-light bands (Fig. 1f). Pairs of moderately electron-dense bands (2 nm wide) alternated with single denser bands (3 nm) wide with a periodicity of about 13 nm. The reaction of these structures to the stains used indicated that they contained protein, and their form suggested that they might be of crystalline nature. They were not observed in normal vegetative hyphae or sporangia.

Our observations indicate that globose bodies were produced by hyphae when they encountered conditions unfavourable for growth or sporulation. It is relevant to note that some strains of *Dactylosporangium thailandense* have been shown to carry phages which cause only limited and delayed lysis (Higgins & Lechevalier, 1969). These had polygonal heads (75 nm diam), tails (200 nm), and were observed in substrate hyphae but not in globose bodies. It was suggested that they were released slowly from enlarged cells of the hyphae. The presence of a poorly lytic phage also provides possible explanations for some of our observations. The small swellings observed on lysing hyphae (Fig. 1a), may be associated with phage release. The clearly-defined light areas in the cytoplasm of globose bodies resemble phage condensation areas, and in isolated sections phage particles were possibly still in place (Fig. 1d). Phage may also provide an explanation for the crystalline inclusions. Similar structures have been observed in phage-infected cultures of *Actinoplanes* sp. (Wilmoughby, Smith & Bradshaw, 1972), *Clostridium cochlearium* (Pope, Yolton & Rode, 1968), and *Bacillus thuringiensis* (Norris & Proctor, 1969). In *Clostridium* and *Bacillus*, the inclusions consisted of clusters of rods or hollow cylinders and it was suggested that they might be associated with defective phage production.

It seems likely, therefore, that the globose bodies of *Dactylosporangium* are products of abnormal development, induced by phage infection or other factors causing hyphal lysis. They bear some resemblance to swollen structures produced by the actinomycete *Intrasporangium calvum* (Lechevalier & Lechevalier, 1969).

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