Fine Structure of the Spore Sheath in *Streptovorticillium* Species

By T. CROSS AND R. W. ATTWELL

*Postgraduate School of Studies in Biological Sciences,
University of Bradford, BD7 1DP, Yorkshire*

AND R. LOCCI

Istituto di Patologia Vegetale, Università di Milano, 20133, Milan, Italy

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Actinomycetes bearing whorls and umbels of short spore chains at regular intervals on their long aerial hyphae and originally included in the genus *Streptomyces* were later reclassified as a separate genus *Streptovorticillium* (Baldacci, 1958, Baldacci, Farina & Locci, 1966). However, these two genera share a number of common morphological, developmental and physiological characteristics which have prevented several workers from accepting their separate generic status (e.g. Krasilnikov, 1970). The similarities have also appeared to discourage the further study of streptovorticillia and it has been assumed that other properties are shared.

During an extensive scanning electron microscope study of the morphology of *Streptovorticillium* species (Locci & Petrolini Baldan, 1971) it was noticed that all showed twisted umbel hyphae which later became subdivided into the chains of arthrospores. No true spirals were observed and when viewed in silhouette by means of the transmission electron microscope the individual spores have appeared smooth (Shirling & Gottlieb, 1968*a, b*, 1969) and lack the conspicuous warts, spines and hairs (Küster, 1955) or knobs (Lyons & Pridham, 1971) which ornament certain *Streptomyces* species. These surface structures develop from the spore sheath (Arai & Kuroda, 1962; Rancourt & Lechevalier, 1964) which also characteristically bears many short and randomly arranged elements termed fibres or rodlets (Vernon, 1955; Hopwood & Glauert, 1961; Dietz & Mathews, 1962; Wildermuth, 1970, 1972*a, b*; Enquist & Bradley, 1971; Wildermuth, Wehrli & Horne, 1971; Williams, Bradshaw, Costerton & Forge, 1972). The fine structure of the *Streptovorticillium* spore sheath was examined to explain the twisted form of the umbel hyphae and the lack of appendages.

METHODS

Early studies concentrated on *Streptovorticillium griseovorticillatus* ISP 5507 but were later extended to include many of the species described by Locci, Baldacci & Petrolini Baldan (1969). The sporulating aerial hyphae grown on inclined coverslips embedded in Czapek's agar were coated with gold palladium under vacuum (Locci & Petrolini Baldan, 1971) and examined with a Stereoscan electron microscope (Mark 2A, Cambridge Instrument Company, Cambridge). Spores were also viewed in silhouette using an AEI EM6B transmission electron microscope and the methods recommended for the International *Streptomyces* Project (Shirling & Gottlieb, 1966).

Fig. 1. *Streptoverticillium griseoverticillatus*. (a) Scanning electron micrograph showing twisted umbel hyphae. (b) Transmission electron micrograph showing a detached portion of the spore sheath. (c) Transmission electron micrograph of spores enclosed within sheath.

RESULTS AND DISCUSSION

The aerial hyphae of *Streptoverticillium griseoverticillatus* showed the characteristic twisted form of the umbel branches prior to the appearance of a beaded spore-chain outline (Fig. 1a). The chain of spores was surrounded by a thin sheath which contained a con-

spicuous and continuous helical thickening (Fig. 1*b*). Segments of the sheath still invested individual spores when the latter separated. Numerous parallel sheath fibres, in the form of a multiple helix, gave the illusion of basketwork when segments were viewed in transparency (Fig. 1*c*) after the shrinkage of the enclosed spore. Individual fibres were about 10 nm in diameter and at least 10 such fibres were involved in the helical pattern.

We have seen this regular helically thickened sheath in all representative species chosen from the 12 *Streptovercillium* series described by Locci *et al.* (1969). Careful examination of *Streptovercillium* spore electron micrographs in earlier papers confirm this to be a common feature of the genus (e.g. *Streptovercillium baldaccii* in Farina & Locci, 1966; *S. mashiensis* in Shirling & Gottlieb, 1968*b*).

It is postulated that the amorphous material of the sheath or the fibrous thickening contracts during spore maturation or during preparation for scanning electron microscopy, so imparting the twisted form to the sporulating hyphae. The characteristic appearance of the *Streptovercillium* sheath, which is quite unlike that described for the several *Streptomyces* species already examined, supports the separation of these organisms into distinct genera. The absence of short rodlets from the sheath may also explain the absence of conspicuous surface appendages for it has been suggested that these subunits appear to aggregate to form the spines and hair-like projections (Wildermuth, 1972*a*; Williams *et al.* 1972).

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