SPORE ULTRASTRUCTURE IN SPOROTHRIX SCHENCKII

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Abstract

Pathogenic strains of *Sporothrix schenckii* may show triangular spores, whose angular shape is maintained by a tie-beam effect in the inner cell wall structure. This difference in wall structure lies adjacent to a folded and possibly more active part of the spore cytoplasm. The supposed generation of asci in old cultures was simulated by the death of hyphae which are reinvaded by intrahyphal growth with intrahyphal spore production, while true asci were not seen.

Introduction

A triangular shape of *Sporothrix schenckii* spores is associated with a heavier pigmentation and a more regular pathogenicity of the organism. Another type of spore, of smaller size and oval outline, is sometimes found enclosed in groups within a capsule in older cultures, and their status either as ascospores or endospores has been debated. Observations on these topics have hitherto mostly been made upon South African strains, from which our isolates also are derived, and which constitute the subject matter of our ultrastructural study.

Material and methods

An unusual case of disseminated sporotrichosis of skin and bone provided the fungus used in our study. The early isolates from subcutaneous abscesses yielded a filamentous phase with the classical small oval spores, while 9 months later an isolate from a granulomatous cyst in the patella was intensely pigmented with large oval and triangular spores. (In the figures these are described as early and late isolates respectively.) With this change in behaviour of the fungus the clinical responsiveness to potassium iodide was lost, intravenous miconazole proved valueless, and cure was obtained with amphotericin-B and limited surgery.

After 30 days' growth on Sabouraud dextrose agar with actidione and chloramphenicol, spores were suspended and fixed in 5% phosphate-buffered gluteraldehyde at pH 7.3 for 8 hours and spun down. The pellet was consolidated with a drop of agar, washed overnight in phosphate buffer, and post fixed in 1% phosphate-buffered osmic acid. Following ethyl alcohol dehydration, with propylene oxide transition to imbedding in Spurr's epoxy resin, sections were cut on an L. K. B. microtome with glass knives, stained with uranyl acetate and lead citrate and examined under a Philips 301 electron microscope.

Results

**Triangular spores.** When these spores tumble about in currents of fluid, one can appreciate microscopically that they are purse-like and flattened when viewed along the edges. To describe them as conical spores is not correct. Ultra-thin sections are therefore bound to pass through triangular spores in planes which often do not suggest triangularity, but they do not recall conic sections in their full variety. The point of spore attachment comprises one of the three angles, while the other two arise on the circumference during spore growth. The ultrastructural detail follows.

**The cell walls** (Fig. 1) of spores and conidiophores have a thin dense outer layer and a thicker more translucent inner layer. The outer layer is nearly twice as thick over the spores as on the hyphae despite running continuously over both. It splits off by springing away like a recurved calyx.
when ruptured. Where the spores detach, it leaves a collar-like rim on the spore side of the fracture line.

The inner layer of the spore, which is mostly about three times thicker than the outer, is the most durable part of the fungus structure. Young spores just budded from the conidiophore are thinner walled with a diffuse granularity and little differentiation of the wall into layers. The common ovoid spores show a more translucent inner layer, while the mature large and triangular spores have granular, flocculent, streaky and laminated deposits in them with translucent zones in between.

At the point of attachment which comprises the basal angle in a triangular spore, the spore side of the thickened inner cell wall appears dense, contrasting sharply with the conidiophore 'denticle' which is a pale convex bulge of the hyphal inner cell wall. At the two outer angles of the triangular spore as seen in fortunate sections, the inner cell wall also exhibits shoulder-like thickenings. These evidently act as a tie-rod which angulates the spore profile, and this site also shows a regional increase in the dense deposits (Fig. 2).

The cytoplasm (Fig. 3) of the younger spores is clearly structured but later becomes densely obscured. Nuclear lobes are occasionally clearly visible and tend to be basal. Mitochondria are also seen but sometimes they leave only ghost-like relics. Other intracytoplasmic structures are of uncertain nature – annular spaces with peripherally radiating extensions, various droplets, curled structures etc. – artifacts, degenerations, storage or special structural and metabolic features being indistinguishable among these.

The plasma membrane is the site of some particular features. In younger spores it is closely interlocked with the inner cell wall and is difficult to distinguish from the cytoplasm. With further spore growth, the plasma membrane mostly appears as a smoother single layer along the straighter sides of the spore, but retracts at the angles