Reserve substances in *Paxillus involutus* sclerotia

Determination by histochemistry and X-ray microanalysis

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**Summary.** The ectomycorrhizal fungus, *Paxillus involutus*, produces sclerotia in culture. These can be induced to form on agar medium by exposing mycelium grown at 25°C to various temperatures between 6°C and 15°C. Sclerotia formed at 10°C and above were large and covered with drops of exudate, while those formed at 6°C or 8°C were very small and did not produce an exudate. Mature sclerotia were bounded by a compact rind and contained abundant storage reserves. Histochemistry of the larger sclerotia showed large quantities of protein stored as protein bodies in the cytoplasm, lipid present as small droplets, glycogen granules stored in the cytoplasm and polyphosphate present as small granules in the cytoplasm and in the protein bodies. Energy dispersive X-ray microanalysis confirmed the presence of phosphate in the granules and was used to map its distribution throughout the sclerotium. The smaller sclerotia induced at 8°C and below on the same medium had the same basic structure and composition, but lacked the complex phenolic cell network found in large sclerotia, and had abundant extracellular polysaccharides. The rind was not well developed and these small sclerotia are interpreted to have been arrested at an early stage of development.

**Keywords:** Sclerotia; *Paxillus involutus*; Histochemistry; X-ray microanalysis; Protein bodies; Polyphosphate.

**Introduction**

Sclerotia are resting structures produced by many fungi. They survive conditions unfavourable for growth of mycelium (Willett 1971) and germinate to produce mycelium, asexual spores or sporocarps bearing sexual spores (Coley-Smith and Cooke 1971). A number of ectomycorrhizal fungi produce sclerotia and, because of their capacity for long term survival, these are potentially useful in artificial inoculation programmes (see Grenville et al. 1985 b; Fox 1986 a, b). As with any resting structure, survival and infectivity of sclerotia depend on the amount and types of reserves present and consequently there has been much interest in the nature of the storage material and its location in various types of sclerotia. Insoluble reserves reported from histochemical work include glycogen, extracellular carbohydrate, polyphosphate, lipids and protein (see Kohn and Grenville 1989 a, b). The importance of protein as a reserve in sclerotia of several fungal species has been confirmed by the extraction of large amounts of either one or two high molecular weight proteins which, together, form a very high proportion of the total protein in the sclerotium and are not detected in vegetative mycelium (see Novak and Kohn 1988, Antibus 1989).

Recently, it has become clear that the sclerotia of many fungi store a major amount of their protein reserves as membrane-bound protein bodies (Bullock et al. 1980 b; Russo and van Etten 1985; Backhouse and Stewart 1987, 1988; Newstead and Huner 1988; Kohn and Grenville 1989 a, b). These are reported generally to be homogeneous structures which stain with anionic dyes, such as amido black and Coomassie blue at acid pH. The first sclerotial protein bodies described, those in *Sclerotinia minor*, were reported to stain green with toluidine blue (Bullock et al. 1980 b), a reaction characteristic of phenols and attributed to the aromatic amino acid content. Recently there have been two reports of pink metachromatic staining with toluidine...
blue O in protein bodies (Backhouse and Stewart 1987, Kohn and Grenville 1989 a). This was attributed by Backhouse and Stewart (1987) to acidic protein. Protein, however, does not stain with toluidine blue at the pH used by these authors and this indicates that another molecule may be present together with the protein in the protein body matrix. This study reports on the nature of the metachromatic material found in the matrix of sclerotial protein bodies in the ectomycorrhizal fungus Paxillus involutus, a member of the Agaricales. Sclerotia are also known to store large numbers of metachromatic granules which tend to be located primarily in the cortex (see Kohn and Grenville 1989 a). These granules are reported to increase in number early in differentiation of the sclerotia of S. minor (Bullock et al. 1980 b) and, together with the protein reserves, they are degraded and utilised during germination (Bullock et al. 1983). The granules in S. minor have been tentatively identified as polyphosphate by their histochemical staining reactions, but it has not been confirmed in this, or any other sclerotium, that they do contain phosphorus. In microorganisms, metachromatic granules generally occur either as single large, or multiple small bodies within otherwise transparent vacuoles, not containing protein. This applies to actively growing mycelium as well as sclerotia (Bullock et al. 1980 b) and other aggregated fungal structures, such as the sheath of ectomycorrhizal fungi (Ashford et al. 1975, 1986; Ling Lee et al. 1975). In contrast, in higher plants, storage of phosphorus is often associated with storage of other macromolecules, as for example in seeds where phosphorus, stored as inositol hexaphosphate (phytin), occurs as globoids within protein bodies (Lott and Spitzer 1980). The occurrence of similar metachromatic, globoid-like inclusions in the protein bodies of P. involutus sclerotia is reported here. Confirmation that metachromatic granules contain phosphorus and therefore are likely to be polyphosphate is obtained by X-ray microanalysis. A description of the general structure and reserves of these sclerotia is also given.

Materials and methods

Materials

Paxillus involutus (Batsch.) Fr. isolate No. 0262 obtained from Dr. J. A. Fortin, Université Laval, Sainte Foy, Québec, was grown on Modified Melin Norkrans medium (Marx and Bryan 1975) solidified with 1.2% agar. Sclerotia were produced by exposing plates of mycelium grown at 25 °C for 2 weeks to temperatures between 6 °C and 14 °C for 8 weeks. Mature black sclerotia of differing sizes, were removed from the colonies grown at 10 °C, cut in half and fixed in 2% glutaraldehyde in HEPES buffer (Massicotte et al. 1985). Material was dehydrated in a graded ethanol series, infiltrated with LR White resin which was polymerised at 60 °C and sections were cut at about 1μm with a Porter Blum microtome. Histochemical reactions and energy dispersive spectroscopy (EDS) analyses were carried...