ARTHROSPORE DIFFERENTIATION IN A CLINICAL STRAIN OF TRICHOPHYTON MENTAGROPHYTES

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Trichophyton mentagrophytes var. granulare is one of the most common dermatophytic fungi causing ringworm in man. It is generally considered to have three types of asexual spores, microconidia, macroconidia and arthrospores. Of the three types, only arthrospores are found in active ringworm lesions and therefore should be employed during in vitro testing of antifungal agents. The significance of arthrospores has only recently been acknowledged and conditions for production and isolation studied (Bibel et al 1977; Hashimoto & Blumenthal 1977). With the intention of testing the susceptibility of arthrospores of Trichophyton to antifungal compounds, the problems encountered using reported methods of production in this species were investigated. The methods essentially consisted of: broth culture (Emyanitoff & Hashimoto 1979); aqueous suspension on agar (Hashimoto & Blumenthal 1977; Weigl & Hejtmanek 1979); dialysis membrane sandwich culture on agar (Emyanitoff & Hashimoto 1979); spore suspension in broth on agar (Bibel et al 1977; Hashimoto & Blumenthal 1978).

A clinical isolate of T. mentagrophytes was subcultured on Sabouraud Dextrose Agar at 30°C for 1 to 2 weeks to give abundant microconidia which were then incubated for various time intervals using combinations of temperature, humidity, atmosphere and media. Arthrospore formation, maturity and viability were determined using phase contrast microscopy. Fluorescent and electron microscopy were used to differentiate between the spore forms. The degree of arthrospore formation was measured by counting % hyphal tips containing arthrospores (Emyanitoff & Hashimoto 1979) and arthrospore viability was assessed by germ tube production.

Broth culture was found to give abundant arthrospore formation at 37°C whereas solid media methods gave better results at 30°C. An increased CO_2 tension has been recommended for arthrosporogenesis (Bibel et al 1977). In this particular strain of T. mentagrophytes, an environment enriched with CO_2, either in the form of sodium bicarbonate in a liquid culture of gaseous CO_2 at a concentration of 8-20%, appeared to be necessary for abundant arthrospore formation. Arthrospores were not produced in the sandwich culture method although an enriched CO_2 atmosphere was present, suggesting that the correct microenvironment may not have been achieved between the two layers of cellulose dialysis membrane. Saturated humidity was present during arthrospore formation, however, maturation and disarticulation occurred more readily on solid media or in a broth overlay culture which had partially dried out, although further dessication reduced arthrospore viability. Dry conditions and a CO_2 enriched atmosphere promoted formation of macroconidia which was not reported in the methods examined. The strain used in this study required a longer incubation period, particularly to mature, than that found in other reports. This investigation has highlighted the problems of obtaining a mature and viable arthrospore population free from other spore forms. An aqueous suspension on agar gave best results for this strain.

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