Mycologia, 91(6), 1999, pp. 1102-1103

Direct mechanical dispersion and in vitro culture of fusiform rust fungus single basidiospores[1]

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Abstract: Single basidiospores of *Cronartium quercuum* f. sp. *fusiforme* were cast from telia suspended over a solidified nutritional medium affixed to an operating orbital shaker. Spores thus mechanically dispersed and isolated, germinated to develop single-genotype colonies.

Key Words: axenic, basidiomycete, fusiform rust, mycelium, single genotype

Axenic culture of the otherwise obligatively parasitic rust fungi generally require a high spore density for colony establishment. In the case of *Cronartium quercuum* f. sp. *fusiforme,* the fusiform rust fungus (FR) of Southern pines, frequency of colony establishment from basidiospores was related directly to inoculum density (Amerson et al 1985). The sole instance of FR colony development from a single isolated basidiospore on freshly prepared medium (Frampton 1984) could not be repeated, although later success was realized using a nurse medium (Hu and Amerson 1991). In order to preclude the need for this protracted, multistep method, an extremely simple technique was developed to disperse single basidiospores from telia directly onto a freshly prepared medium, for development of single-genotype cultures. This technique may be modified easily for use with macroscopic sporophores of other fungi.

In the earlier method of Hu and Amerson (1991), colonies first were established from multiple spores cast from telia onto cellulose nitrate membranes overlying nutrient medium. Growth of these colonies "conditioned" that medium for later nutritional support of individual spore germination and hyphal growth. These single spores were inoculated following collection in germination-inhibitory pH 2.2 water during 36-48 h darkness. Spore concentration was determined, and inoculations were made to the nurse medium from which the membrane had been removed, using appropriate spore dilutions.

In the method reported here, telia were cleaned by manipulation through agar-solidified water as described by Amerson and Mott (1978), then applied lengthwise to the surface of a double-sided, strongly adhesive tape (Stanley Products, New Britain, Connecticut), which had been affixed to the inner surface of a petri dish (85 X 15 mm) lid. Telia were selected from those showing ongoing teliospore germination. One or more telia were applied to one exposed adhesive surface of the tape. The petri dish lid from which the tape and telia were suspended, then was replaced over the agar-solidified medium (Hu and Amerson 1991). The medium as prepared had been modified by the use of (i) Oxoid Proteose Peptone® (Unipath Ltd., Basingstoke, Hampshire, England) as the peptone-of-choice, and (ii) 1% Sigma agar (Sigma Chemical, Co., St. Louis, Missouri) as the gelling agent. The plate was sealed with Parafilm. A second piece of double-sided tape was applied to the base of that petri dish. Using the second adhesive surface of that tape, the dish was applied with hand-pressure to the platform of an orbital shaker (Lab-Line Instr., Inc., Melrose Park, Illinois), such that the entire closed petri dish bearing telia firmly suspended over the nutritional medium, tightly adhered to the shaker platform. The shaker was operated at room temperature (20-22 C) for 4 h at 480 rpm in a 1 cm orbital radius.

During that time, basidiospores were cast peripherally from the suspended telia, and distributed singly onto the medium. Plated medium thus inoculated, was inverted and dark-incubated at room temperature for 2 d, then examined under the microscope for both germinated FR basidiospores and the presence of any contaminants. It was found that a l-h shaking provided several hundred widely-distributed basidiospores, on medium over which 6-8 telia were suspended. Longer periods on the shaker increased spore inoculum density to an unnecessary or excessive level. Germination of single spores was infrequent, a possible result of spore exudates inhibitory to germination (Spaine and Kaneko 1993). Of those that germinated, colony establishment was rare (less than 1%), though numbers of colonies that developed were adequate for further research. Colony development was slow; growth to a 5 mm diam colony occupied 2 mo, in contrast to the one month required from the collective spores from a telium. The

Accepted for publication June 29, 1999.

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mycelial network was diffuse, similar to that reported by Hu and Amerson (1991).

Only three contaminant microorganism colonies developed in 25 plates inoculated in this fashion. The contaminant colonies easily were excised from the agar medium. The telia were not dislodged during the shaking. Indeed, the thick adhesive surface of the tape to which the telia had been affixed showed signs of having smoothed or flowed during the period of shaking, slightly burying the telia at their edges.

It is possible that use of a nurse medium would have increased the frequency of colony development from single spores. However, adequate numbers of colonies were obtained in this fashion. Developed colonies could be easily cut to small segments and subcultured to develop dense mycelia on freshly prepared, chemically identical medium.

The direct relationship of inoculum density to colony establishment is common. Nutritional media contrived for in vitro growth of naturally obligate parasites are considered minimally adequate for axenic growth. Thus, an unnaturally large inoculum of spores may initiate an early supportive level of complex substrate catabolism. High inoculum density may support colony establishment in more than one way. Extracellular enzymes of fungi are important for fungal pathogen establishment in the living host, as well as for utilization of potential nutrients in the environment (Chang et al 1992). Additionally, fungal synthesis of plant growth regulators (PGR) or PGR-like compounds may be selfstimulatory to growth. Several such PGRs have been reported to be both products of fungal biosynthesis and stimulatory to fungal growth (Nakamura et al 1978, Kawanabe et al 1985).

The technique described here may be used in cases where the sporophore or sporophore-bearing tissue is (i) adequately small to remain adherent to the tape during the vigorous shaking used to distribute the spores, and (ii) yet large enough to resist complete submersion in the flowing thick adhesive during shaking. Additionally, the tissue- source of the spores must be surface-disinfested of opportunistic contaminants, which may be difficult in cases of large segments of sporophore-bearing tissue, such as the basidiocarp of a mushroom. However, this has proven straightforward with telia of the fusiform rust fungus. This method offers an easy, one-step way to obtain single-genotype fungal colonies directly from their spore source.

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