Tryblidiella rufula in artificial Culture[1]

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(With plate)

Introduction

In studies of collections of *Tryblidiella rufula* on diverse host substrate Muthappa (1967) discussed the taxonomic position of this fungus. In the same collections a pycnidial fungus belonging to *Haplosporella* was observed in association with the developing ascocarps. Seshadri (1967) reported an association of *Haplosporella* pycnidium with ascocarps of *T. rufula* but without proof of their relationship.

Earlier, Shear (1933) and Voorhees (1939) reported the development in culture of *Diplodia*-like conidia in association with microconidia of unknown origin produced in pycnidia followed by the production of an ascigerous stage typical of *Tryblidiella*. Baumeister (1957) on the other hand reported the development of a phoma-type of pycnidial locules in cultures of *Eutryblidiella hysterina*. Occurrence and association of species of *Haplosporella* and *Diplodia* were not uncommon in hosts naturally infected by *T. rufula*.

It was thus clear that no definite information was available on the conidial status of *Tryblidiella rufula* as the results so far reported were widely variable and often conflicting. It was, therefore, decided to undertake cultural studies to determine the conidial status of this fungus, if any.

Materials and Methods

Fresh ascocarps of *T. rufula* growing on dead branches of *Citrus sinensis* Osbeck., were softened under water. A dilute suspension of ascospores was obtained in blanks of sterile water, 10 ml of which was poured on 2% water agar in petri plates. The ascospores germinated in less than 24 hours (Fig. 1). Single germinating ascospores were marked out under microscope, lifted, and planted in fresh plates containing various media. Mono-ascospore cultures thus obtained were grown on the following media in petri plates of uniform diameter with uniform

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quantity of the medium. The various media employed in this study were (1) potato dextrose agar, (2) potato dextrose agar with bark extract, (3) potato agar, (4) dextrose agar, (5) cornmealagar, (6) M_2 agar : NaCl — 20 grams, yeast extract — 10 g, glycerine — 12 ml, glucose — 10 g, KH_2PO_4 — 0.1 g, $MgSO_4$ — 0.12 g, $FeSO_47H_2O$ — trace, $CuSO_4$ — trace, $CaSO_4$ — 0.5 g, bacto-agar — 50 g, distilled water — 2000 ml.

The mono-ascospore cultures of this fungus made good growth on all of the media used except potato agar and dextrose agar. PDA, cornmeal agar and M_2 agar supported good growth as well as sporulation. M_2 agar was found to be particularly favorable for the development of pycnidia and ascocarps probably because of the B-complex vitamin contents of this medium in the form of yeast extract.

Formation of Pycnidia

Submerged formation of pycnidia was obtained in all the six media used, at the end of ten days, pycnidial development was, particularly, profuse along the margins of the colony. In M_2 agar, however, pycnidia developed uniformly throughout the medium (Fig. 2). Sections of such pycnidia revealed, under the microscope, production of profuse conidiophores lining the entire wall layer (Fig. 3). Pycnidia measured 160—220 $\times$ 128—192 µ. Conidiophores were 7.2—12.6 $\times$ 1.8 µ. Microconidia were hyaline, round to oval, thin-walled and borne on simple as well as branched conidiophores in wall layers (Fig. 4). Repeated attemps to germinate these microconidia failed indicating that the microconidial locules were in the nature of spermogonia. Periodic observations of these cultures for a period of over two months did not reveal the development of any conidial stages.

Ascigerous Stage

Of the six different media employed in these experiments the production of well developed ascocarp of *T. rufula* was obtained in cornmeal agar and M_2 agar at the end of sixty days under laboratory conditions. The ascocarps produced mature ascospores characteristic of the original fungus. The production of ascocarps was, however, scanty in these media as compared to the microconidial locules. The mature ascocarps, asci and ascospores produced in culture were, however, smaller than those obtained from the host as could be seen from the following table.

Comparative dimensions of Tryblidiella rufula obtained from host and artificial culture

Substrate	Ascocarp	Asci	Ascospores
1. Host	1—2 $\times$ 0.5—1 mm	126—150 $\times$ 10—12 µ	25—28.5 $\times$ 9—10.5 µ
2. Culture	0.75—1 $\times$ 0.5	120—140 $\times$ 10 µ	20—24 $\times$ 8—8.5 µ

Except for the smaller dimensions of ascocarps, asci and ascospores

obtained in culture the other characters such as shape, color and septation of ascospores, bitunicate nature of asci, discoid form of ascocarps and numerous pseudoparaphyses were typical of the original fungus on host.

Discussion

T. rufula was successfully grown in single ascospore cultures accompanied by the production of microconidial locules containing microconidia in young cultures followed by the development of well developed ascigerous stage of the fungus. Production of conidia of *Diplodia* sp. was not observed as reported earlier by Shear (1933) in *T. hysterina* and Voorhees (1939) in *T. rufula* and *T. fusca*. The constant association of *Haplosporella* with *T. rufula* under natural conditions as observed by Seshadry (1967) appears to be of an accidental nature without any specific genetic relationship. The occurrence of microconidial locules in culture is suggestive of spermatisation as a possible mode of sexual reproduction in this fungus. The production of ascigerous stage in single ascospore cultures proves this fungus to be homothallic.

Summary

Tryblidiella rufula was successfully grown in single ascospore cultures with the production of microconidial locules followed by the development of mature ascocarps typical of the fungus. No conidial stage was obtained in culture. The fungus is homothallic.

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Explanation of Plate

Fig. 1. Germinating ascospores. — Fig. 2. Mature asci and ascospores in culture. — Fig. 3. Cross section of a pycnidium in culture: A = pycnidium, B = conidiophores, C = conidia.