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A New Technique for Single Spore Isolation of Two Predacious Fungi Forming Constricting Ring

Kalika Prasad Singh*, Dharmendra Kumar and Pinaki Bandyopadhyay

Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221005, India
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A new technique for single spore isolation was developed for predacious fungi forming constricting rings directly on the spores using *Dactylaria brochopaga* and *Arthrobotrys dactyloides*. Constricting rings were induced directly on the spores by transferring the spores in 25 ppm solution of DL-Valine in sterile distilled water. Freshly hatched and thoroughly washed second stage juveniles of *Meloidogyne incognita* were transferred into cavity blocks containing induced rings for trapping and killing of nematodes. The killed nematodes were surface sterilized with streptomycin and inoculated into petri dishes containing maize meal agar media with 100 ppm streptomycin. The petri dishes were incubated at 29±1°C for few days which yielded axenic culture of these fungi.

KEYWORDS: ***Arthrobotrys dactyloides*, Constricting ring, DL-Valine, *Dactylaria brochopaga*, *Meloidogyne incognita*, Predacious fungi**

Pure cultures of fungi are usually made by single spore isolation or hyphal tip method (Tutte, 1969), however at times it becomes difficult to locate a single spore on to a medium under stereoscopic binocular microscope. Similarly hyphal tip method also leads to complexity in selection of single hypha if the fungal growth is dense and compact. For single spore isolation of some predacious fungi which produce constricting rings directly on the spores in presence of nematodes, soil and an amino acid DL-valine (Drechsler, 1937; Mankau, 1962; Barron, 1977; Singh and Bandyopadhyay, 2001; Kumar, 2003), a new technique has been developed to isolate these fungi using trapped and killed nematodes by induced rings. This technique is convenient and easier as compared to the conventional techniques mentioned above. This paper describes a new technique for single spore isolation of *Dactylaria brochopaga* and *Arthrobotrys dactyloides* forming constricting rings directly on the spores.

The idea was to induce direct rings on the spores of *A. dactyloides* and *D. brochopaga* by 25 ppm DL-valine and use such spores for trapping of second stage juveniles of *Meloidogyne incognita* by single conidial trap and transfer a trapped nematode on maize meal agar medium for growth of these fungi. The colony developed on the medium would be essentially from a single spore only. The technique was perfected using spores of *D. brochopaga* and *A. dactyloides*. These fungi were isolated from compost by the method described by Duddington (1955). The fungi were grown in pure culture on maize meal agar medium (Maize meal-20 g, Agar-20 g, water-1000 *ml*). For single spore isolation, about 100 spores of each fungus were harvested from 10 day old cultures with a sterile fine needle and transferred separately into cavity blocks (50 mm × 50 mm) each containing 0.5 *ml* of 25 ppm of DL-Valine in sterile distilled water. The cavity blocks were incubated for 15~18 hr at room temperature (25~30°C) for induction of constricting rings directly on the spores (Fig. 1a). Freshly hatched second stage juveniles of *M. incognita* were collected in large number from the egg masses of infected brinjal roots. The nematodes were concentrated to be around 500 juveniles per ml in sterile distilled water. The juveniles were washed at least 5 times with sterilized distilled water and a small drop containing nearly 50 nematodes were transferred into cavity block containing induced rings. The cavity blocks were incubated at room temperature (25~30°C) for 24 hours for trapping and killing of nematodes. The captured and killed nematodes each by a single ring of a spore (Fig. 1b and c) were collected in cavity blocks containing solution of 0.5 *ml* of 100 ppm streptomycin in sterile water. The captured nematodes were washed and surface sterilized at least four times with 100 ppm streptomycin in sterile distilled water. A single juvenile trapped and killed by a single ring formed on a single conidium was picked up with the help of a flame sterilized fine needle and transferred into sterilized petri dishes containing thin layer of maize meal agar medium containing 100 ppm streptomycin. Several such trapped and killed nematodes were transferred singly in separate petri dishes. The petri dishes were incubated at 29±1°C. Within 24 hours, the mycelium of the predacious fungus grew outside the nematode body (Fig. 1d and e). After 5~7 days the fungus grew well in petri dishes and produced spores (Fig. 1f). Further purification was done by transferring conidia from a single conidial

*Corresponding author <E-mail: kpsmppbhu@rediffmail.com>

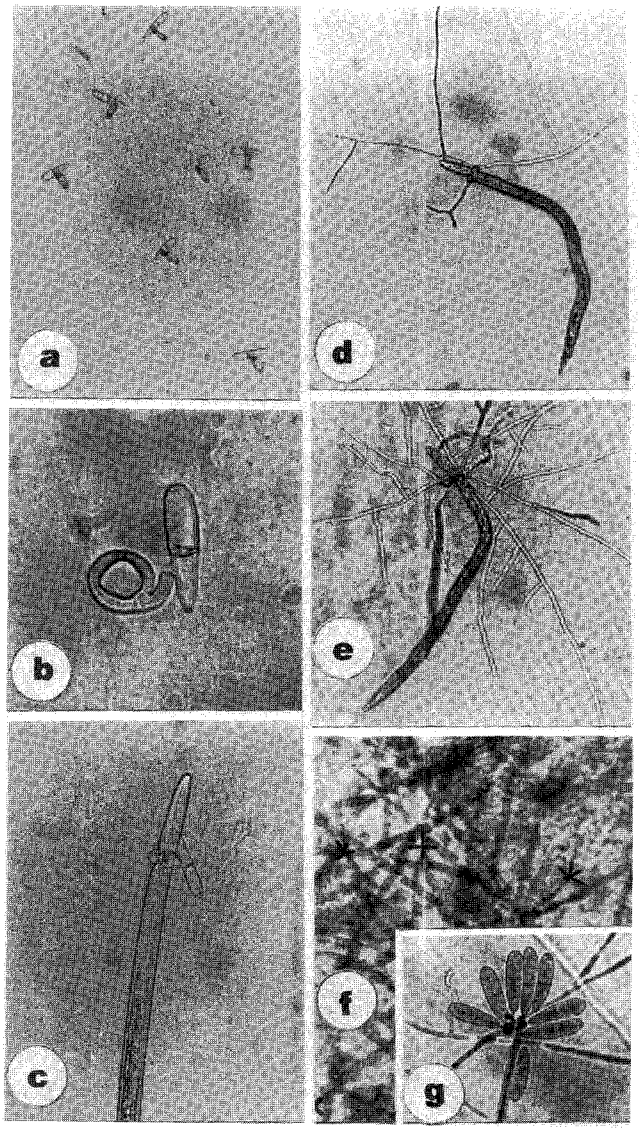

Fig. 1. a~g : Single spore culture of *Arthrobotrys dactyloides* from second stage juvenile of *Meloidogyne incognita* trapped and killed by a constricting ring directly formed on the spores. **a**. Constricting rings directly formed on the spores in response to valine (×125). **b**. Enlarged view of constricting ring directly formed on the spore of *A. dactyloides* (×600). **c**. Second stage juvenile of Meloidogyne incognita trapped by constricting ring formed on the spore of *A. dactyloides* (×250). **d & e**. Hyphal growth arising from the constricting ring or spore cell of *A. dactyloides* (×125). **f**. Development of conidial heads of *A. dactyloides* on medium (×70). **g**. Enlarged view of conidial head of *A. dactyloides* (inset) (×300).

head. The experiment was repeated at least 20 times and every times it yielded the axenic culture of both the predacious fungi separately.

During single spore isolation besides clear visibility of a spore in a stereoscopic microscope, a spore may or may not germinate after transfer in a medium. However, when a nematode is trapped and killed by a single ring formed on a conidium, the hyphae penetrate and grow into the nematode body. Further hyphal growth outside the nematode body is initiated from inflated cells of the ring or spore cell onto the medium. In *A. dactyloides* the external hyphae develop from the spore cells after trapping of nematodes (Kumar, 2003) whereas, in *D. brochopaga* the hyphae develop from the inflated cells of the constricting ring involved in the capturing of a nematode (Singh and Bandhyopadhyay, 2001) This is only because the fungus is already in the growth phase due to availability of nutrition from nematode body. Thus there are cent percent chances of recovery of the fungus from the trapped and killed nematodes. Growth of the tested fungi was not inhibited after incorporation of the streptomycin at the range of 50~150 ppm concentrations into the medium which is broad spectrum and effective in eliminating the bacterial contamination. Use of streptomycin was also suggested by Tutte (1969) for checking bacterial contamination during isolation of a fungus. The developed technique is precise and comprehensive for single spore isolation of predacious fungi forming constricting rings directly on the spores and is superior to single spore isolation or hyphal tip methods because it ensures picking up of a single spore through a trapped nematode. Picking and transfer of such trapped nematodes is much easier under a stereoscopic microscope. The technique is also superior because the growth of the fungus from a single spore occurs without failure due to availability of nutrition from the nematode. The technique is useful for researchers working on predacious fungi for biological control of plant parasitic nematodes.

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