



A simple and reliable method for obtaining monoconidial culture and storage of *Magnaporthe oryzae*

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ABSTRACT

Monoconidial isolates of *Magnaporthe oryzae* were successfully cultured and maintained using a simple, reliable and inexpensive isolation procedure developed by us. It can also be performed with minimum equipment. This procedure will be helpful for researchers to save their time in standardizing the isolation and storage. We have isolated 72 *M. oryzae* monoconidial isolates from different agro climatic regions of Karnataka, India and pure cultures were stored in filter papers, in our investigation. The current procedure is useful in carrying out research investigation requiring genetically uniform cultures.

Keywords: *Magnaporthe oryzae*; Monoconidial isolate; Rice blast disease

INTRODUCTION

Rice blast pathogen *Magnaporthe oryzae* B.C. Couch, (Anamorph: *Pyricularia oryzae* Cavara) (Couch and Kohn, 2002) is considered as the highest loss causing fungus in the world (Dean *et al.*, 2012; Kihoro *et al.*, 2013) because of its worldwide distribution and destructiveness. This pathogen is genetically highly polymorphic having greater adaptability to change in the environment. Development of effective screening technique based on the basic understanding of pathogen biology, epidemiology and identification of disease resistant cultivar is needed to eradicate this disease. Establishment of pure culture is mandatory for researchers for understanding virulence of this pathogen. This can be achieved initially through single spore isolation from the disease sample. There are many methods such as spore dilution technique with various modifications, micro-manipulators which are available to obtain monoconidial cultures where special microscopes are used to pick up a single spore.

Single spore isolation is the first step to study the virulence spectrum and molecular aspects of a given pathogen. Establishment of pure culture of

M. oryzae is a challenging task due to the cross contamination of other fungi. There is a necessity for complete step-wise procedure to isolate rice blast pathogen. Many researchers around the world are trying hard to establish pure culture with a little or incomplete information on isolation procedure of this pathogen (ISTA, 2014). The objective of this paper is to present a detailed procedure in a stepwise manner to obtain pure culture of *M. oryzae*, so that researchers can use this simple and reliable technique for single spore isolation. The resultant cultures are genetically uniform as they are established from single conidium.

MATERIAL AND METHODS

Isolation of the pathogen, culturing and maintenance of pure culture

1. The blast fungus *M. oryzae* was isolated from blast infected plant parts such as leaves, neck, collar, node, stem and panicle.
2. Diseased samples were cut into small pieces around the infected area showing the blast lesion including the edge of the lesion (1-2 cm) and then subjected to surface sterilization with 1% sodium hypochlorite for 1:30 min followed by 3 washes with sterile distilled water.
3. These infected plant pieces were fixed in the upper half of Petri dish lined with moist filter paper. 10ml of sterile water is transferred to the lower half to create 100% relative humidity within the moist chamber (Fig. 1A).
4. Infected plant pieces were also placed on glass slides which were kept inside moist chamber. Set up was incubated at 28°C for 48hrs to enhance sporulation. After incubation, these infected plant pieces were examined under stereo binocular microscope to confirm the typical elliptical or spindle shaped *M. oryzae* spores.
5. After confirmation the plant pieces were taken in Eppendorf tube with 1 ml sterile autoclaved water and inverted to mix and 100 µl of diluted conidial suspension (4×10^1 per ml) was spread uniformly using spreader (L-rod) on 2% water agar and incubated overnight at 25°C (Fig. 1B).
6. The Petri plates with germinating conidia were placed in inverted position without opening the lid and were observed under 10X. The position of germinated

conidium was marked with the help of a marker pen (Fig. 1C). Later a single germinating spore was picked up from the marked region of the Petri plate and was transferred to a fresh cornmeal rice straw agar plate with streptomycin sulphate (40mg/L) and incubated at 28°C for 14 days (Fig. 1D). This step was carried out inside the laminar air flow.

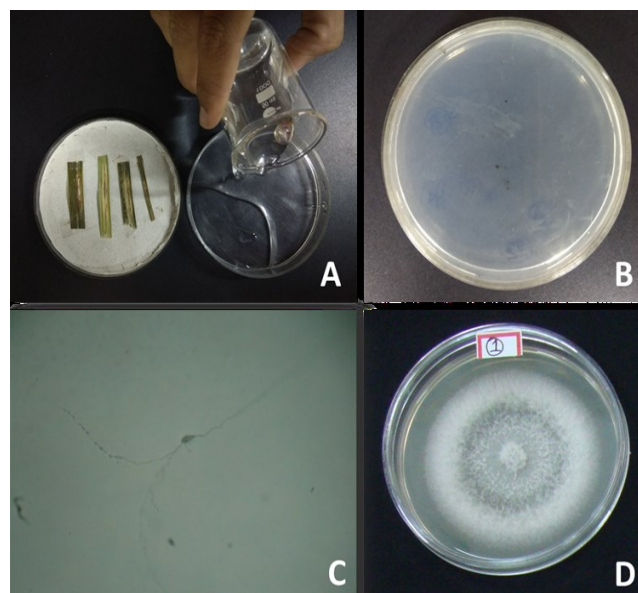


Fig. 1: (A) Preparation of Moist Chamber, (B) 2% Water agar plate, (C) A view of germinated spore in 10X, (D) 14 day old *Magnaporthe oryzae* monoconidial culture in oat meal agar.

Long-term storage of *M. oryzae*:

1. Whatman filter papers were cut in to small pieces of about 15mm square. These filter papers were put in a Petri dish and sterilized.
2. These sterile paper disks were placed on the surface of Oat Meal Agar (OMA) plate, then inoculated with a block of 0.5cm diameter *M. oryzae* using transfer needle (Fig. 2A). Petri dishes were incubated at 28°C for 4-5 days i.e., until the filter paper was covered by the fungal mycelium (Fig. 2B).
3. These colonized filter papers were kept inside sterilized Petri dishes which were dried by placing them in desiccators at room temperature for 4-5 days (Fig. 2C & D).

These dried filter papers with fungal mycelium were cut in to small pieces of about 3-5mm square and were stored in microcentrifuge tubes which in turn were kept in storage boxes at -20°C (Fig. 2E & F).

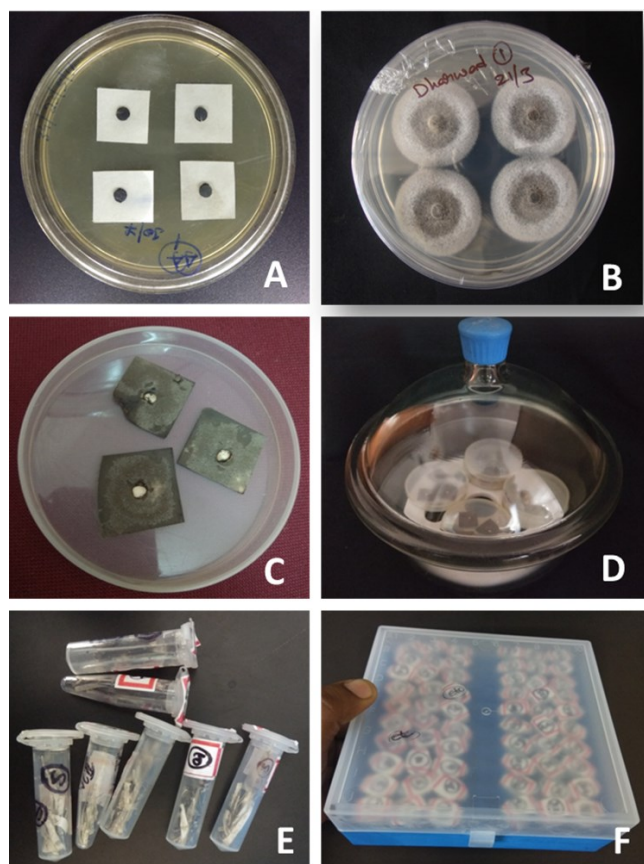


Fig. 2: Steps followed during long term preservation of *Magnaporthe oryzae*. (A) Sterile filter papers were placed on the surface of Oat Meal Agar plate with 0.5cm diameter *Magnaporthe oryzae* culture blocks on it, (B) 6 day old *Magnaporthe oryzae* culture on filter papers. (C) Separated filter papers taken to fresh petri plates for drying. (D) Dried filter paper with mycelium in desiccators, (E) Filter paper was cut in to small bits and taken to sterile eppendorf tubes, (F) View of storage box.

RESULTS AND DISCUSSION

Long term preservation of pure form of fungi is essential for the researchers to carry out investigation with genetically uniform population. Maintaining viability and the stability of the microbial cells should be taken in to consideration during preservation of fungal cultures (Castellani, 1939). This is possible when cultures are established using single conidium to obtain genetic uniformity throughout the cultures. These cultures are useful in studying variations among the isolates. In the current study a total of 72 mono conidial isolates were isolated from blast infected plant parts collected from 11 different districts of Karnataka for studying variability.

Our mono conidial isolation procedure is simple, inexpensive, can be performed with minimum equipment and does not need expert technicians to carry out. A similar procedure was followed during isolation of *Fusarium verticillioides* and *Fusarium subglutinans* fungi (Aboul-Nasr and Abdul, 2014). However, the storage steps are not given here. Isolation steps are not given completely in most of the publications available for *Magnaporthe oryzae*. In one of the studies carried out in Tanzania, *Pyricularia oryzae* was obtained to understand the pathogenic variation where complete protocol was not given for the isolation and storage of rice blast fungus (Chuwa *et al.*, 2013). In one of the research papers, isolation of single spore using a hand-made glass needle was found to be useful, but this technique has limitations because the researcher should be skillful in making ideal glass needles. In addition to this he should have sufficient practice and patience (Goh, 1999). In the present investigation sequential steps are explained so that any researcher is able to establish monoconidial culture with ease.

When a diluted conidial suspension was spread on water agar, distance between the spores increased and it was easy to pick the separated individual germinated spore using transfer needle. In case of micro drop spore suspension method, only 50% of micro drops contained a single spore and 35% contained no spores (Ho and Ko, 1997). In the present method all spores in the plate were located, picked up and transferred to fresh medium to obtain mono conidial cultures. Hence this method has the advantage of being easy to locate well separated spores for mono conidial isolation. The method is better than the technique reported by Fukuta *et al.*, (2009). In their JIRCAS Working Report, direct picking of single conidium was shown. The technique described by them has a few limitations such as: i. Conidia isolated may be viable or non viable ii. Conidia may be damaged during picking and transferring iii. Chances of picking more than one conidium during transfer.

During long term storage of *M. oryzae*, the purity and viability of all the 72 isolates were found preserved in dry filter papers with in the eppendorf tubes for a period of 4 years. Our method is easier to perform with minimal equipment, low cost and easy of storage when compared to cryogenic systems (Buell and Weston, 1947; Chandler, 1994; Ingroff *et al.*, 2004).

CONCLUSIONS

The Present procedure is on the pathogen infecting a staple food crop namely, rice. An understanding of the pathogen dynamics at molecular level is necessary for implementation of strategies for management of the disease. Monoconidial isolation is required for maintaining genetic uniformity throughout the culture for this purpose.

During the current investigation we have developed a step wise detailed protocol for monoconidial isolation and storage of pure culture of *M. oryzae*. This will come in handy for many researchers who are working in this line. This procedure will supplement the researchers and rice breeders for developing rice blast resistance cultivars. This procedure can be well adopted for monoconidial isolation of other species of *Magnaporthe*.

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Compliance with ethical standards

This article does not contain any studies with human or animal subjects.

Competing interests

The authors declare that they have no competing interests.

REFERENCES

- Aboul-Nasr MB and Abdul A (2014) A simple technique for single spore isolation of *Fusarium verticillioides* and *Fusarium subglutinans*. *World J. Biol. Sci.*, 2(1): 021-025.
- Buell CB and Weston WH (1947) Application of the mineral oil conservation method for maintaining collection of fungal cultures. *Am. J. Bot.*, 34 (10): 555-561.
- Castellani A (1939) The viability of some pathogenic fungi in sterile distilled water. *J. Trop. Med. Hyg.*, 42:225-226.
- Chandler D (1994) Cryopreservation of fungal spores using porous beads. *Mycol. Res.*, 98(5): 525-526.
- Chuwa CJ, Mabagala RB and Reuben MS (2013) Pathogenic variation and molecular characterization of *Pyricularia oryzae*, causal agent of rice blast disease in Tanzania. *Int. J. Sci. Res.*, 4(11): 1131-1139.
- Couch BC and Kohn LM (2002) A multilocus gene genealogy concordant with host preference indicates segregation

of a new species, *Magnaporthe oryzae*, from *M. grisea*. *Mycologia.*, 94(4): 683-693.

- Dean R, Van Kan JA, Pretorius ZA, Hammond Kosack KE, Di Pietro A, Spanu PD and Foster GD (2012) The top 10 fungal pathogens in molecular plant pathology. *Mol. Plant Pathol.*, 13(4): 414-430.
- Espinell-Ingroff A, Montero D and Martin-Mazuelos E (2004) Long-term preservation of fungal isolates in commercially prepared cryogenic microbank vials. *J. Clin. Microbiol.*, 42(3): 1257-1259.
- Fukuta Y, Vera C and Kobayashi N (2009) Development and characterization of blast resistance using differential varieties in rice. JIRCAS Working Report, (63).pp 127.
- Goh TK (1999) Single-spore isolation using a hand-made glass needle. *Fungal Divers.*, 2:47-63.
- Ho WC and Ko WH (1997) A simple method for obtaining single-spore isolates of fungi. *Bot. Bull. Acad. Sin.*, 38: 41-44.
- ISTA (2014) Detection of *Pyricularia oryzae* on *Oryza sativa* (Rice). International rules for seed testing annexe to chapter 7: Seed health testing methods. International seed testing association (ISTA), Bassersdorf, Switzerland, pp. 7-11.
- Kihoro J, Bosco NJ, Murage H, Ateka E and Makihara D (2013) Investigating the impact of rice blast disease on the livelihood of the local farmers in greater Mwea region of Kenya. *SpringerPlus*, 2: 308.