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CHARACTERIZATION OF SOIL INHABITING FUNGI *MORTIERELLA CAPITATA* IN PAKISTAN

NASIR AHMED RAJPUT[1,*], MUHAMMAD ATIQ[1], ABDUL MUBEEN LODHI[2], REHANA NAZ SYED[2], AKHTAR HAMEED[1], OWAIS IQBAL[2,3] AND BABAR KHAN[1]

[1]*Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan*
[2]*Department of Plant Pathology, Sindh Agriculture University Tandojam, Pakistan*
[3]*Department of Plant Pathology, Yunnan Agricultural University, Kunming, Yunnan, China*
**Corresponding author's email: nasirrajput81@gmail.com*

Abstract

Mortierella capitata was isolated from chilli filed soil by the survey of microbial pathogens in the chilli field from different places of Hyderabad, Pakistan. *M. capitata* was identified based on morphological characters and phylogenetic analysis of the internal transcribed spacer (ITS-1 and ITS-4) sequence results. These results indicated that the species of *Mortierella* has not previously been reported from Pakistan

Key words: Chilli filed, ITS sequence, Zygomycota, Hyderabad.

Introduction

During survey of fungal communities, species is a genus of *Mortierella* isolated from soil samples collected in chilli field. *Mortierella* species are soil inhabiting fungi belonging to the order Mortierellales within the division of Zygomycota. First species *Mortierella polycephala* was identified from a mushroom by Coemans in 1863, and as the name of genus *Mortierella* was specified in honor to M. Du Mortier (Coemans, 1863). *Mortierella capitata* Marchal is one of more than 100 species of *Mortierella* that can be existing as saprophytes in any substrate, rhizosphere of fresh water and soil (Marchal, 1891; Degawa, Gams, 2004; Yadav *et al*., 2014; Nguyen, Lee, 2016; Leon *et al*., 2017). Several soil inhabiting fungi belonging to the *Mortierella* genus have been recognized as producers of polyunsaturated fatty acids and other arachidonic acid (Kendrick, Ratledge, 1992; Botha *et al*., 1999; Wang *et al*., 2011). Moreover, *Mortierella capitata* strain RD000969 was also produced nervonic acid accounting for up to 6.94 percent of its whole cellular fatty acid, which was produced a large amount of long-chain monounsaturated fatty acid and small amount of ultralong-chain fatty acid (Umemoto *et al*., 2014), overall 99 percent of nervonic acid was localized at the (*sn*-1, 3) location of triacylglycerol in fungal cell. *M. capitata* known as the rare fungus, which was isolated from Fluvo-aquic soils that were help to increase chlorophyll, gibberellic acid and biomass content in maize crop (Li *et al*., 2020). *M. capitata* has excellent capacity to affect the degradation rate of straw, which may help to alter the structure of the native microbial community (Tamayo-Velez & Osorio, 2018).

Our studies indicated that a species, *Mortierella capitata* was isolated from soils collected in Hyderabad, Pakistan, during the survey of microbial pathogens from the soil in chilli field. The objective of this work was to conduct morphological and molecular analyses to characterize unrecorded species, *M. capitate* Pakistan.

Materials and Methods

Isolation of fungi from soil samples: Soil samples in polythene bags were collected from various locations of Hyderabad, Sindh, Pakistan in 2016. The soil sample for each location were taken from 15–18 cm depth, removed surface waste and stored at 4°C. Serial dilution method was used for fungus isolation (Wakesman, Fred, 1922) on water agar (WA) supplemented with 50 µg/ml of penicillin and streptomycin sulfate for 6–7 d incubated at 28°C and single transferred to PDA plates for pure cultures.

Identification of isolated fungi: Morphological characters of the isolated fungi were observed on WA culture and the plates were incubated 28ºC for 7 d. *Mortierella* species were identified based on the structure of sporangia, zygospore, chlamydospores and sexual structures as described by (Mehrotra *et al*., 1963; Embree, 1963; Chalabuda, 1968; Linnemann, 1969; Milko, 1974; Gams, 1977; Watanabe, 1990; Degawa, Tokumasu, 1997). Morphological structures were obtained from the isolates NAR-Mc1 and NAR-Mc2 and observed under a compound microscope. The photographs were taken from the Nikon Eclipse 80i microscope with digital camera DS-Ri1 (Nikon Corporation Japan).

DNA extraction and PCR amplification: A CTAB rapid fungal genome DNA extraction kit (Biomiga, Inc. San Diego, CA 92121) was used to extract total genomic DNA from isolates NAR-Mc1 and NAR-Mc2 followed by instructions (Bellemare *et al*., 2018). Amplification of ITS region (internal transcribed spacer) was conducted and sequenced with the primers (ITS-1, 5′-TCC GTA GGT GAA CCT GCG G-3′) and (ITS-4, 5′-TCC TCC GCT TAT TGA TAT GC-3′) (White *et al*., 1990). Polymerase chain reaction (PCR) was conducted and take 50 µl reactions, including 50 ng of DNA as template, 10 µM gene primers, 5 µl 10 × reaction buffer, 10 mM dNTPs, 1.25 µl rTaq and 36.5 µl of deionized water. Amplification of PCR was conducted in thermal cycler under the following program: 5 min at 95ºC followed by

30 cycles at 95ºC for 40 s, 60ºC for 40 s, 72ºC for 50 s and the last step was carried out at 72ºC for 10 min. PCR products were purified and sequenced at the TSINGKE Biological Technology, Kunming, China with the same primers. Two new sequences of both isolates NAR-Mc1 and NAR-Mc2 are deposited in GenBank (NCBI).

Phylogenetic analysis of nucleotide sequence: Amplification of two PCR products was cut from agarose gel (2%) and purified by using the TaKaRa MiniBEST Agarose Gel DNA Extraction kit (Kusatsu, Shiga, Japan) according to instructions of Manufacture Company. For sequence analysis, both purified DNA samples were sent to the TSINGKE Biological Technology, Kunming China. Sanger dideoxy sequencing was used to determine the sequences of purified DNA samples. Both detailed reference sequences that were retrieved from GenBank database to evaluate the phylogenetic relationship. Alignment of two sequences were compared with *M. capitate* sequences which were previously published and available in GenBank using Bioinformatics tools such as MEGA 7 (Kumar *et al*., 2016).

For phylogenetic studies, the ITS sequence along with different *Mortierella* species accession numbers of sequences retrieve from GenBank database were used to construct a phylogenic tree by using program Kimura 2-parameter substitution model for neighbor-joining tree construct (Kimura, 1980). The robustness of phylogeny tree was examined through the nearest-neighbor with 1,000 bootstrap replicates.

Results and Discussion

Morphological characteristics of *M. capitata* isolates: During the present studies, two isolates NAR-Mc1 and NAR-Mc2 of *M. capitata* were isolated from chilli field soil in Hyderabad, Sindh, Pakistan. We examined the morphological characters of NAR-Mc1 and NAR-Mc2 isolates were almost the similar. Isolates produce light dirty whitish growth on PDA with the growth rate of 8.1–8.4 cm incubated at 28°C for 6 d. The mycelial growth was dirty color, cottony, concentric pattern produced and mostly zonate (Fig. 1A). Microscopic characters were observed in WA culture. Septate mycelium branched with coenocytic complex and sporulation was abundant (Fig. 1B–D). The thick-walled chlamydospores were observed with globose and 16–34 µm in length (Fig. 1E, F). Sporangiophores were arising from the mycelial branching are the size of 30–350 µm long, hyaline and erect, tapering from the maximum width of 12–32 µm at the bottom and 3–8 µm at the top, sporangium bearing single terminal (Fig. 1G–K). Spores were measured 3.5–4.5 × 3.2–4.5 µm globose to subglobose (Fig. 1L, M). The morphological characteristics of *M. capitata* agreed with the taxonomic status (Degawa, Tokumasu, 1997; Leon *et al*., 2017).

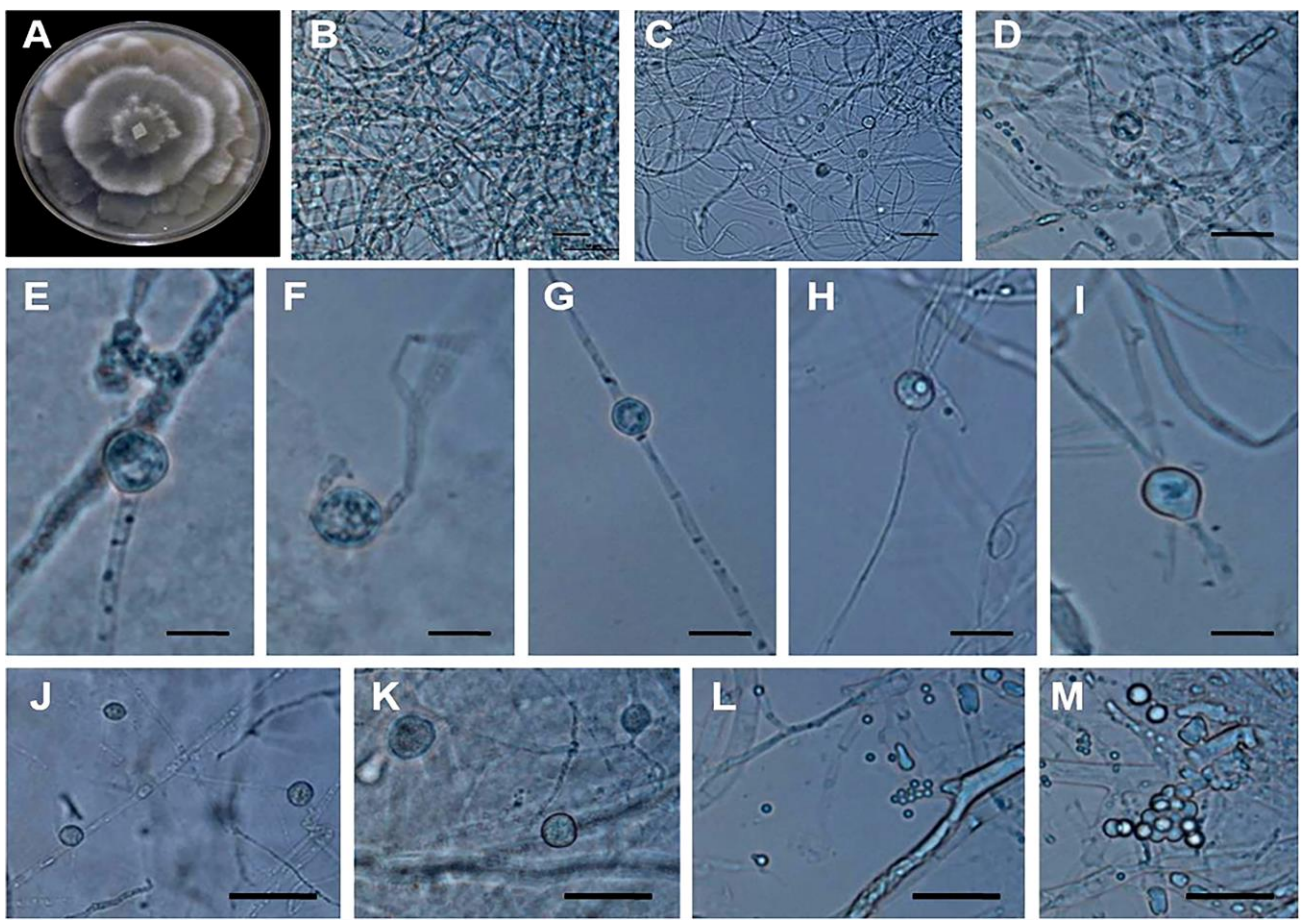

Fig. 1. Morphological characteristics of *Mortierella capitata* isolate NAR-Mc1. A colony growth in potato dextrose agar plate; B mycelial hyphae; C, D: branching of sporangiophore; E, F: chlamydospore; G–I, mature sporangia with hyphae; J, K: sporangiophore with terminal sporangia; L, M spores on water culture B, C 5 µm; D–I 10 µm; J–M 20 µm.

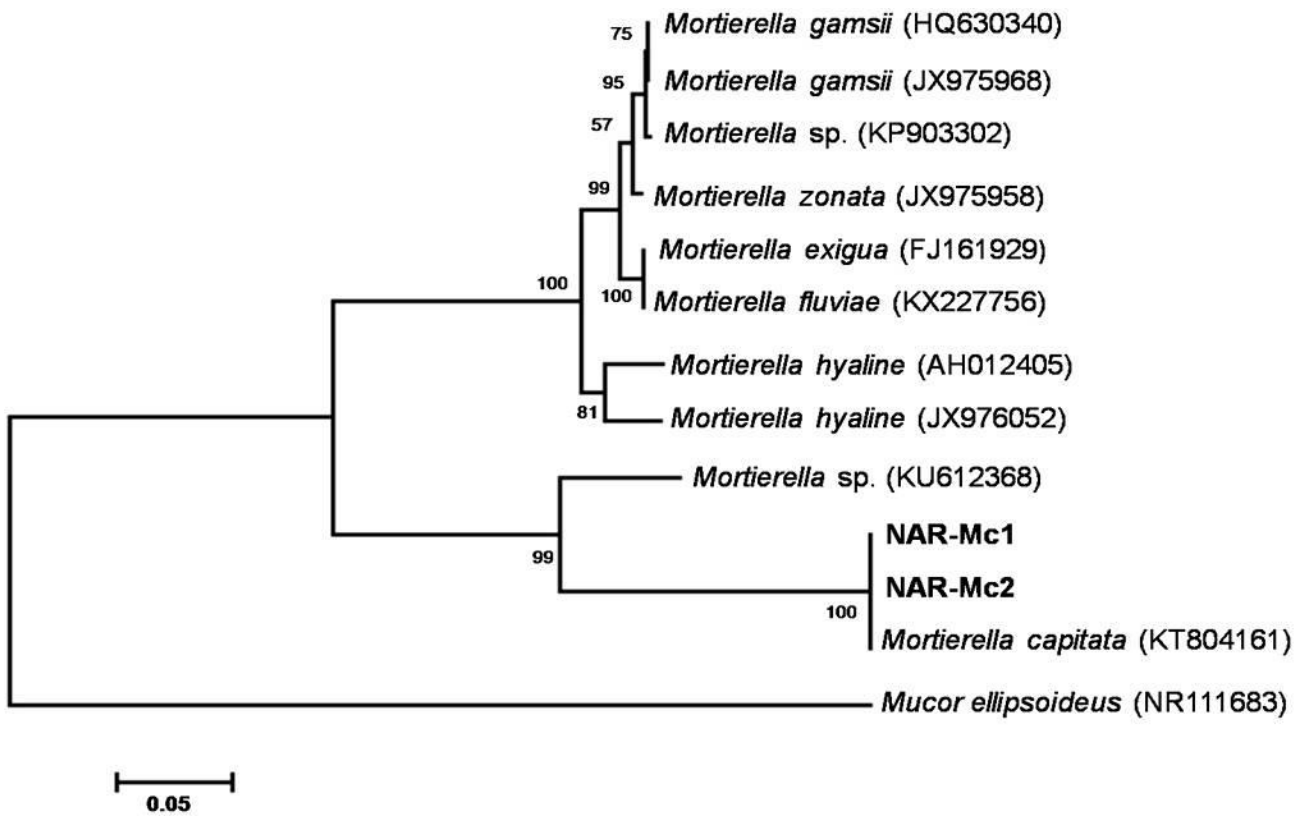

Fig. 2. Phylogenic tree structure by Neighbor-joining algorithm using Kimura-2-parameter, for *Mortierella capitata* NAR-Mc1 and NAR-Mc2 and reference sequences based on maximum likelihood analysis of the partial ITS rDNA sequences. Sequence of *Mucor ellipsoideus* was used as the outgroup. Numerical values of ≥50% on branches are the percentage of 1,000 bootstrap replicates that support the branch. The scale bar represents the number of substitutions per site.

Molecular phylogeny analyses of *M. capitata* isolates: Two amplified DNA sequences of the ITS rDNA region of the two isolates NAR-Mc1 and NAR-Mc2 were submitted to NCBI GenBank under the accession numbers MF115610 and MF115611**,** respectively. Next, to determine the phylogenetic relationship was derived from comparisons of the thirteen ITS gene sequences and a dendrogram was constructed with *Mucor ellipsoideus* (NR111683) as an outgroup species. Isolates NAR-Mc1 and NAR-Mc2 sequences MF115610 and MF115611 are grouped as one cluster and are closely related to *M. capitata* sequences (KT804161) of NCBI database with bootstrap value of 100% (Fig. 2). In conclusion, the taxonomical properties and phylogenetic analysis of isolates NAR-Mc1 and NAR-Mc2 indicated it to be *M. capitata*. Generally, *Mortierella vesiculosa* species are similar as a synonym of *M. capitata* (Linnemann, 1969; Milko, 1974; Gams, 1977). This heterothallic species found in soils inhabited by pillbugs (*Armadillidium vulgare*) (Degawa, Tokumasu, 1997). However, this is the first report of *M. capitata* isolated from soil in Pakistan.

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