Arab Universities Journal of Agricultural Sciences
(2022) 30 (1) 55-59
Website: http://ajs.journals.ekb.eg

Isolation and Identification of Some Egyptian Ectomycorrhizal Sporocarps

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https://doi.org/10.21608/AJS.2022.123225.1470

Received 21 February 2022; Accepted 28 March 2022

Short Communication

Keywords:

Ectomycorrhizae, *Suillus collinitus*, *Protubera* sp., *Pisolithus tinctorius*, Morphoanatomical-based taxonomy, Molecular identification

Abstract: In contrast to the global countries, ectomycorrhizae members are not common in Egypt, however, they are very important in the afforestation of poor lands. Their occurrence in *Pinus* sp., *Clerodendrum* sp., and *Eucalyptus* sp. may help to explain why these trees are the most extensively dominant around the world, especially in Egypt. The identification and characterization of numerous ectomycorrhizal fungi often involve large morphological descriptions of sporocarps alone, which consequently, in some instances, raise arguments about the accuracy of these studies. The present work was achieved to isolate, identify, and characterize three ectomycorrhizal fungi from sporocarps combining morphological and molecular techniques. The morphological characteristics of tested species were assessed and compared to standard taxonomic literature. ITS-rDNA was utilized for molecular analysis using the universal fungal primers ITS1. Identification of these species was confirmed by comparing the sequences of amplified genomes of these species with respective species sequences in GenBank, followed by blast analysis.

1 Introduction

Ectomycorrhizae (ECM) is a mutualistic relationship resulting between distinctive soil fungi and plant roots which are mostly woody trees (Smith and Read 2008). In this relationship, the fungal partner supplies nutrients to plants in exchange for plant carbohydrates produced during photosynthesis (Xie et al 2021). Trees belonging to *Pinus* sp., *Clerodendrum* sp., and *Eucalyptus* sp. present in tropical and temperate regions are reported to harbor a broad variety of ECM fungi (Corrales et al 2018). The majority of ectomycorrhizal fungal species belong to Basidiomycotina, Ascomycotina, and some Zygomycotina (Bai et al 2021).

Ectomycorrhizal symbiotic associations are particularly crucial to plant health and growth as well as enhancing plant tolerance to biotic and abiotic stress conditions (Otgonsuren et al 2016). The ectomycorrhizal fungi have the ability to increase water and nutrient supply to plants, produce enzymes and some types of organic acids, protect plants against soil pathogens and improve soil structure (Prieto et al 2016).

This study aims to isolate and identify some ectomycorrhizal species collected from different Egyptian sites.

2 Material and Methods

2.1 Sporocarps Collection and Characterization

Twenty-three Sporocarps were collected from the Faculty of Agriculture - Ain Shams University, Orman Botanical Garden, Greenhouses Arab Organization for Industrialization and Al-Azhar University, during different seasons to be used for isolation of ectomycorrhizal fungi. Fresh undamaged sporocarps were collected from *Clerodendrum* sp., *Pinus roxburghii* and *Eucalyptus* sp. trees.

The sporocarps were observed to have different characteristics. During transfer to the laboratory, the sporocarps were deposited into waxed paper bags and kept in a cool box. The sporocarps were characterized and preidentified by anatomic-morphological methods, according to Brundrett et al (1996) and Agerer and Rambold (2004-2006).

2.2 Isolation of Ectomycorrhizal Fungi from Sporocarps

The sporocarps were brushed to be free of adhering soil particles and carefully broken to be opened. The isolation method followed that described by Brundrett et al (1996). A small piece of tissue (2 mm^3) was removed with fine forceps and placed on modified Melin-Norkrans medium (MMN) agar media in a Petri dish. Plates were incubated at 25-28 °C. After 3-4 days, samples were observed under a stereomicroscope for initial fungus growth and contamination. In all experiments, pure cultures **(Fig 1)** were maintained in solid MMN at 25±1 ºC and routinely subcultured to fresh medium every 2 months.

2.3 Molecular Identification of Ectomycorrhizal Isolates

Ectomycorrhiza isolates were identified at the molecular level using the universal 18S rDNA primers ITS1 (ITS1, 5′-TCCGTAGGTGAACCTGCGG-3′) according to Amicucci et al (1998).

2.4 DNA Extraction

The mycelial genomic DNA was isolated from 24-day-old cultures of the tested fungi according to the procedure described by Erland et al (1993). The purification step was performed using the Gene Clean II kit (BIO 101 Inc., Vista, CA, USA).

2.5 Selection of Primers

Specific primer pairs for the studied species were selected from the ITS sequences deposited in the GenBank. Moreover, the choice of these pairs of primers was confirmed using the "Oligo" software, version 5 (National Biosciences Inc., Plymouth, MN, USA).

2.6 PCR Amplification Conditions

The amplification reaction was accomplished in a 25 µL mixture volume containing 100 ng of fungal DNA, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1 Triton X-100, 10 mM Tris-HCl pH 8.0, 200 µM each of dATP, dCTP, dGTP, and dTTP, 12.5 moles of primers and 1 U of Taq polymerase enzyme (Promega, Madison, WI, USA). The reaction was performed using the ITS1 primers (ITS1, 5′-TCCGTAGGTGAACCTGCGG-3′) according to White et al (1990). The mixtures were then incubated in a Perkin Elmer Thermal Cycler (model 2400) programmed for 32 cycles, each at 94°C for 15 s, 56°C for 15 s, and 72°C for 40 s. The amplified products were identified on 1% (w/v) agarose gels.

3 Results and Discussions

3.1 Morphological and Microscopic Characteristics of Collected Sporocarps

Despite recent developments in the use of molecular methods for identifying ectomycorrhizal fungi, classical methodologies for studying ECM fungi persist to have numerous advantages. Tracking the characteristics of the sporocarps is regarded as the most dependable way for the identification of ectomycorrhizal fungi. However, molecular taxonomy is not well supported as a preliminary study for identifying fungi using morphoanatomical-based taxonomy. Therefore, using a combination of morphology, anatomy, and molecular characterization is preferred (Bruns et al 1998).

Macromorphological and microscopic features were recorded from freshly collected sporocarps according to Brundrett et al (1996) and Angerer and Rambold (2004-2006); results are presented in **Table 1.** The preliminary identification of the sporocarps were *Suillus* sp., *Protubera* sp., and *Pisolithus* sp.

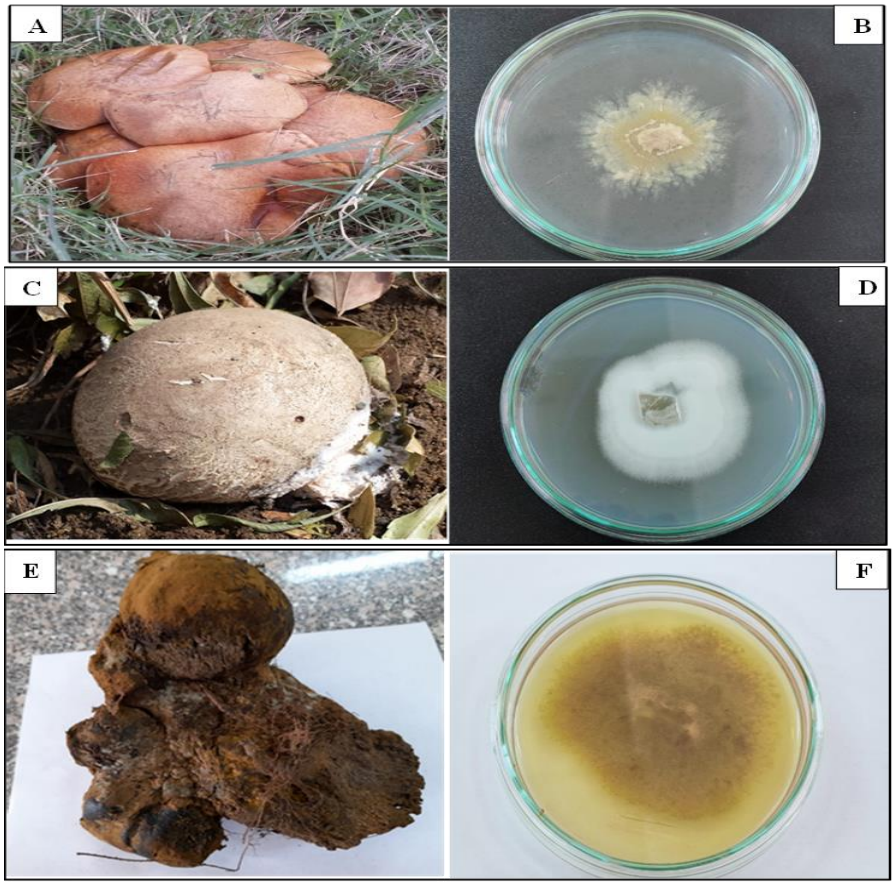

Fig 1. Field photographs of sporocarps and Colony features of isolated strains on MMN agar media of *Suillus collinitus* A and B; *Protubera* sp. C and D; *Pisolithus tinctorius* E and F.

Table 1. Morphological features of collected sporocarps used for general identification

Isolate code / **Characteristic**	**K10**	**M11**	**Pt23**
Host	*Pinus roxburghii*	*Clerodendrum* sp.	*Eucalyptus* sp.
Cap shape	Convex	Egg	Ball
Cap surface	Smooth	Smooth	Smooth
Cap color	Brown	White	Brown
Hymenium	Tubes	Absent	Absent
Stem color	Yellow	Absent	Absent
Stem shape	Cylindrical	Absent	Absent
Gill attachment	Pores	Absent	Absent
Veil ring	Absent	Absent	Absent
Flesh color	Yellow	White	Brown
Spore print color	Brown	Olive	Brown
Spore shape	Ellipsoid	Ellipsoid	Globose
Spore wall	Smooth	Smooth	Echinate
Family	Suillaceae	Phallogastraceae	Sclerodermataceae
Genus	*Suillus* sp.	*Protubera* sp.	*Pisolithus* sp.

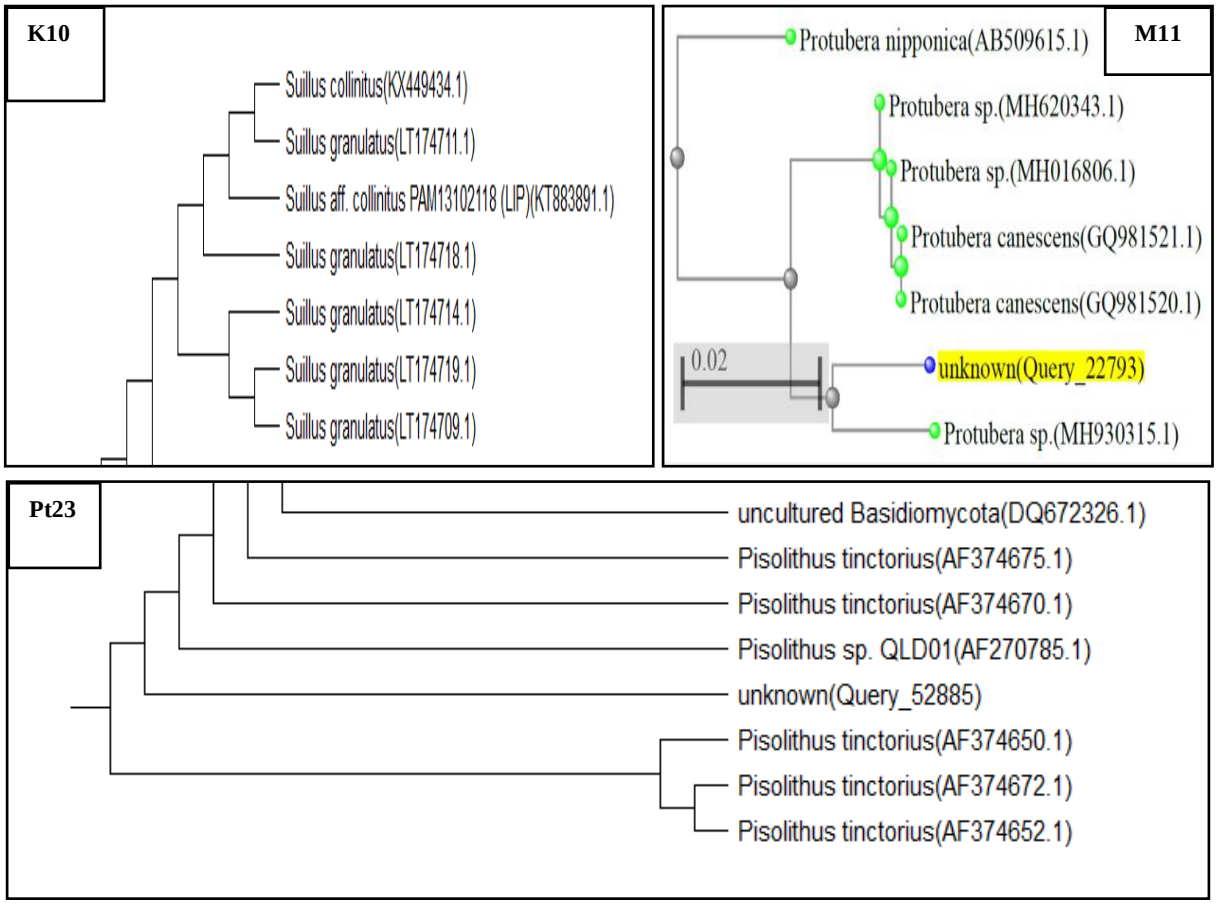

Fig 2. Phylogenetic tree analysis of *Suillus collinitus* (K10), *Protubera* sp. (M11), *Pisolithus tinctorius* (Pt23) based on ITS region gene sequences using specific ITS1 primers

3.2 Molecular Identification of the Morphotyped Ectomycorrhizal Fungi

Genetic analysis of pure cultures using PCR techniques yielded fragments with various sequences and lengths "Fragments length Polymorphism" (RFLP) was subjected to GenBank to be identified.

Taxonomic similarities were assigned to tested species based on BLAST sequence similarity analysis including several most closely matched sequences. The strains have been deposited in the GenBank database and identified as *Suillus collinitus* with a similarity 99.82% and accession number of OL979224:OL979225, *Protubera* sp. with similarity 97.14% and accession number OL998321 and *Pisolithus tinctorius* with similarity 99.31% and accession number of OM125275. The phylogenetic trees of the identified strains obtained from multi-alignment plots of the ITS region are present in **(Fig 2).**

Acknowledgment

The authors would like to express their sincere gratitude to the Microbial Inoculants Center, Faculty of Agriculture, Ain Shams University, for providing financial assistance and laboratory facilities for carrying out the research.

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