



Two new hyphomycete species from petroleum-contaminated soils for mycobiota of Iran

E. Mohammadian ✉

M. Arzanlou

A. Babai-Ahari

Department of Plant Protection, University of Tabriz, Tabriz, Iran

Abstract: During a study on fungal diversity in petroleum contaminated soils in Khuzestan province, two new hyphomycetes were isolated for the mycobiota of Iran. Based on the combination of morphological data and ITS-rDNA sequence, the isolates were identified as *Alternaria obovoidea* and *Emericellopsis pallida*. Morphological descriptions and illustrations are provided for the species. To our knowledge, this is the first report of these two species from petroleum contaminated soils in the world.

Key words: Petroleum pollution, fungal diversity, ITS-rDNA, new record

INTRODUCTION

Soil supports a bewildering diversity of microbes and fauna. Soil microorganisms play important roles in soil development, major biological elements (C, N, P) cycling, the flow of energy, recycling of wastes, and detoxification of environmental pollutants (Aislabie & Deslippe 2013). Fungi are one of the major microbial groups in the soil. Despite the important ecological role of soil fungi, the understanding of fungal community diversity and dynamics in soil is still in demand (De Boer et al. 2005).

Fungi can inhabit diverse ecological niches ranging from normal to extreme environments. The organisms able to grow in extreme environments are generally defined as extremophiles. Extremophiles have been adapted for a number of mechanisms which enable them to tolerate wide ranges of stresses (Palmer et al. 1990; Sterflinger 1998; Gorbushina et al. 2003; Ruibal 2004; Onofri et al. 2007; Gorbushina et al. 2008). There are a large number of physical, chemical and biological stress-inducing conditions in nature, including changes in temperature, osmotic pressure, pH and concentration of water, ions and solutes, as well as exposure to extremes of radiation, pressure and

toxic chemicals, to oxidative conditions and to nutrient starvation (Moye-Rowley 2003).

Some fungal groups have adapted a number of features such as slow meristematic clumpy growth and thick-walled heavily melanised cells which enable them to grow in extreme conditions (Ruibal et al. 2009).

Heterogonous assemblages of fungi inhabit environments contaminated with aromatic compounds. Species in genera *Paecilomyces*, *Verticillium*, *Beauveria* and *Penicillium* species have been reported from petroleum-polluted marine water samples (Fedorak & Westlake 1986). Kim et al. (2010) have reported *Alternaria* species as the most frequently isolated fungi inhabiting creosote-treated wood. In another study, *Aspergillus flavus*, *A. niger*, *A. fumigatus* and *Alternaria* sp. have been isolated from petroleum-contaminated soils (Chaudhry et al. 2012). Zafra et al (2014) also observed *Acremonium*, *Fusarium*, and *Trichoderma* genera from petroleum-contaminated soils. According to Flayyih and Al-Jawhari (2014) *Aspergillus niger*, *A. fumigatus*, *Fusarium solani* and *Penicillium funiculosum* have been isolated from petroleum polluted soil.

In the present study, we describe two new fungal species namely *Alternaria obovoidea* and *Emericellopsis pallida* as new records for the mycobiota of Iran from petroleum contaminated soils in Khuzestan province based on a combination of morphological data and sequence data from ITS-rDNA region.

MATERIALS AND METHODS

Soil samples were collected from petroleum-contaminated soils in oil fields of Khuzestan province, Iran. Samples (approximately 25 g) were collected in depth of 15 cm from top soil. Fungal strains were isolated from soil samples following the protocol of Zhao et al. (2010).

Cultural and microscopic morphological features were examined on potato carrot agar (PCA) (Simmons 2007), malt extract agar (MEA) and oat meal agar (OMA) culture media. Fungal structures were mounted in distilled water and examined at 1000 X magnification using an Olympus-BX41 light microscope. Thirty measurements were taken of the relevant

Submitted 05 Aug. 2016, accepted for publication 19 Nov. 2016

✉ Corresponding Author E-mail: elmo_elham@yahoo.com

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parameters of the conidiophores and conidia. Ninety five percentiles were derived for the measurements with the extremes given in parentheses. An Olympus digital camera system (DP 25) was used to capture high-resolution photographs of microscopic fungal structures.

For molecular identification, in a rapid DNA extraction protocol, a small piece of mycelium scraped from the surface of the plate, then was added to a PCR tubes with 50 μ L TE Buffer, then mashed with a sterile pipette tip or toothpick. The samples were placed in thermocycler at 95 °C for 5 min. DNA extracts were incubated at -20 °C prior to use. The primers ITS1-F, 5'-CTTGGTCATTTAGAGGAAGTAA-3' and ITS4-R 5'-TCCTCCGCTTATTGATATGC-3' (White et al. 1990; Gardes & Bruns 1993) were used to amplify ITS region of rDNA. ITS amplicons were generated for all strains by using the primer pairs ITS1-F and ITS4-R. PCR reactions were performed in 25 μ L volumes containing 1 μ L of template DNA, 2.5 μ L of 10 \times reaction PCR buffer, 1 μ L of 10 μ M dNTP, 1 μ L of each primer and 0.3 μ L of 5.0 U Taq DNA polymerase. Ultrapure water was added to increase the volume to 25 μ L. Amplification of ITS was performed under the following PCR conditions: 95 °C for 5 min, followed by 36 cycles consisting of 94 °C for 45 sec, 57 °C for 30 sec and 72 °C for 90 sec, with a final 10 min extension step at 72 °C. Sequencing was done on a 3730 DNA Analyzer sequencer at Plant-Microbe Genomics Facility (PMGF) at the Ohio State University. The obtained sequences were compared with those included in the GenBank database by using Basic Local Alignment Search Tool (BLAST) in NCBI.

RESULTS and DISSCUSION

Alternaria obovoidea and *Emericellopsis pallida* were identified based on a combination of morphological and sequence data from ITS-rDNA region. These species are reported as new records for mycobiota of Iran. Description of these two species is illustrated below.

Alternaria obovoidea (E.G. Simmons) Woudenberg et al., Studies in Mycology 75: 171-212 (2013)

Basionym: *Ulocladium obovoideum* E.G. Simmons, Mycotaxon 37: 104. 1990. Section Ulocladioides

Colony on PCA attaining a diameter of 35-45 mm after 7 days at 25 °C. Texture woolly, grayish brown, dark blackish brown. Hyphae brown to golden, 4.42 μ m in width as they grew old. Conidiophores brown, golden and curved due to production of conidiogenous cells, 45.26 μ m in length and 4.25 μ m in width. Conidia, solitary, golden or dark brown and punctuate roughened, forming beak 8 μ m in length with 1 to 3 longitudinal and 2-5 transverse septa, subglobose in immature conidia, obovoid to broadly obovoid in those produced at 4-7 days (mature conidia). Conidial

showed an average 32.85 μ m in length and 13 μ m in width (Fig.1).

Alternaria obovoidea has been originally described as *U. obovoideum* however, based on the recent multi-gene phylogenetic analysis a new combination has been proposed as *A. obovoidea* (Lawrence et al. 2013; Woudenberg et al. 2013). *Alternaria obovoidea* can be distinguished from other *Alternaria* species based on morphological characteristics summarized in Simmons (1990, 2007). In this species, conidia are obovoid to broadly obovoid, single or in chains. However, *A. obovoidea* resembles *A. alternata* and *A. arborescens* by producing geniculate conidiophores with secondary conidiophores and fundamentally septate conidia. Conidia of *A. alternata* and *A. arborescens* are smaller than those of *A. obovoidea* (Simmons 1990, 2007).

The identity of this species was confirmed as *A. obovoidea* based on 100% sequence identity of ITS sequence to the type strain of *A. obovoidea* CT4D (GenBank accession KP794083.1); while, substantially deviated from the other species of the genus namely *A. alternata*, *A. arborescens*, and *A. infectoria*. As regards, ITS-rDNA is not useful for robust identification within the *Alternaria* species (Pryor et al. 2002; Andrew et al. 2009; Lawrence et al. 2013); hence, in this study the morphological characteristics were preferred and assigned for identification of *A. obovoidea* species. Pure culture was established using a hyphal tip technique and was deposited in culture collection of Tabriz University (CCTU 1156), and in Iranian Fungal Culture Collection with accession numbers (IRAN 2744C) at the Iranian Research Institute of Plant Protection, Tehran, Iran.

Emericellopsis pallida Beliakova, Mikologiya i Fitopatologiya 8: 386 (1974)

Colonies on OMA reached to 65 mm diameter after 10 days. MEA with slower growing, showed 45 mm diameter after 10 days. Colonies on OMA were white-pink to salmon-colored. Hyphae hyaline, branched, septate had 1-3 μ m wide. Conidiophores macronematous, simple, sometimes lateral branched, septate, smooth-walled, 25-45 \times 2-3 μ m, and borne a terminal phialide. Conidiation abundant, conidia aseptate, hyaline, ellipsoidal, oval, smooth-walled and 3.5-6.0 \times 1.8-2.2 μ m gathering in colorless slime at the tips of the phialides, chlamydospores absent (Fig. 2).

The genus *Emericellopsis* belongs to the order Hypocreales (Sordariomycetes, Hypocreomycetidae), includes 22 species and varieties derived from various ecological niches (Lumbsch & Huhndorf 2007). Currently, *Emericellopsis* comprises saprobic cleistothecial species with acremonium-like conidiation (Crous et al. 2004). *Emericellopsis* species can be distinguished based on a combination of morphological and molecular data (Zuccaro et al. 2004; Duc et al. 2009; Grum-Grzhimaylo et al. 2013).

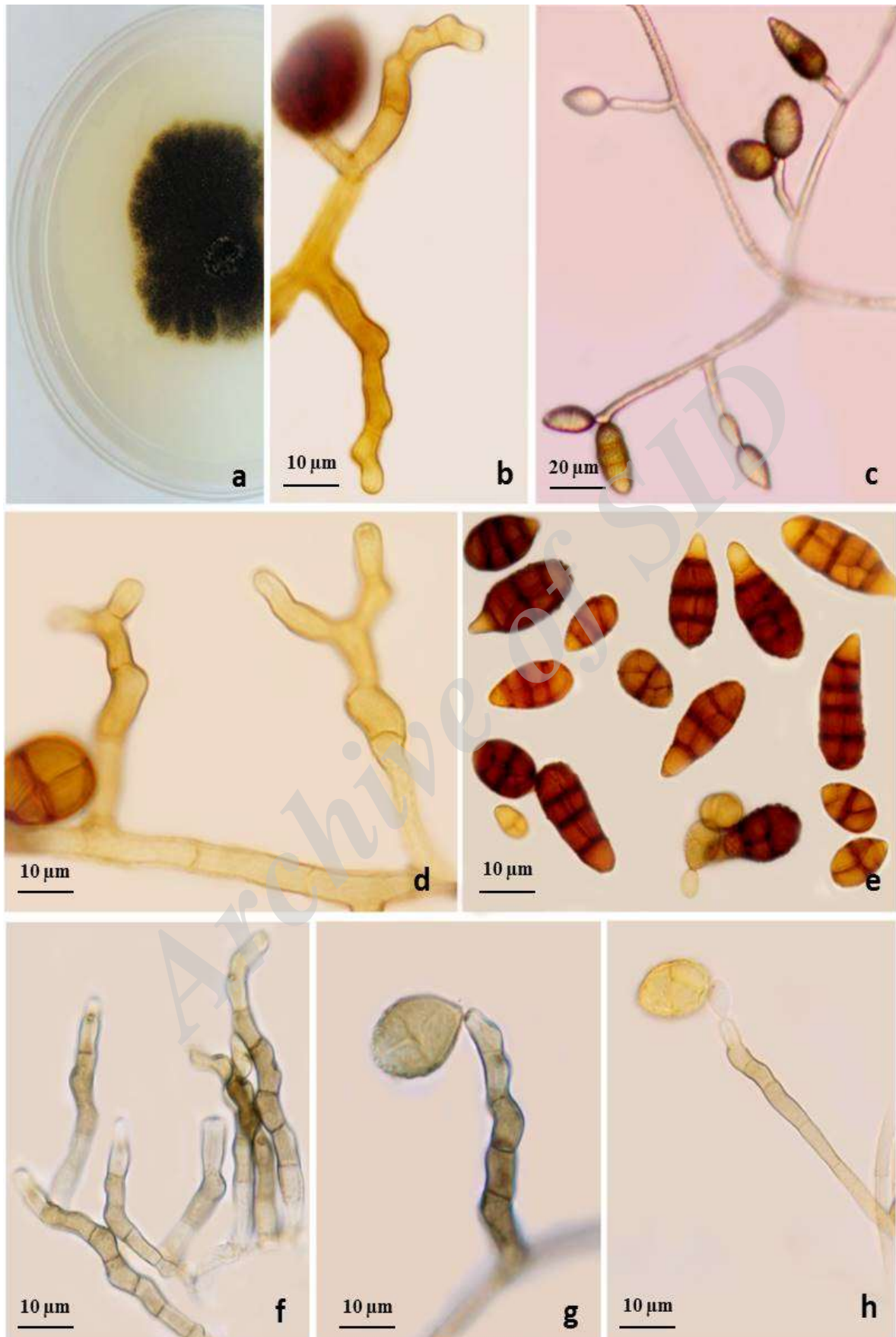


Fig. 1. *Alternaria obovoidea*. a. 7-d-old colony on PCA; b-f. Conidiophores and conidia; g-h. Sporulation.

Morphological features of our isolate are in full agreement with original description of *E. pallida*. The identity of our isolates was further confirmed as *E. pallida* based on 99% identity to the type strains of *E. pallida* XJURML-3 (GenBank accession EU045572.1); while deviated from the sequence data for other *Emericellopsis* and *Acremonium* species available in GenBank namely *E. terricola*, *A. zonatum* and *A. sclerotigenum*. This species is distinguished from other *Emericellopsis* and *Acremonium* species by the differences in conidial shape, sizes, conidiogenous structures and colony characteristics (Crous et al. 2004; Zuccaro et al. 2004; Lumbsch & Huhndorf 2007; Grum-Grzhimaylo et al. 2013). Pure culture was established using a hyphal tip technique and was deposited in culture collection of the University of Tabriz (CCTU 1132), and in Iranian Fungal Culture Collection with accession numbers (IRAN 2782C) at the Iranian Research Institute of Plant Protection, Tehran, Iran.

The sequences are available in Genbank with accession numbers KY053135 and KY039291 for *A. obovoidea* and *E. pallida* respectively.

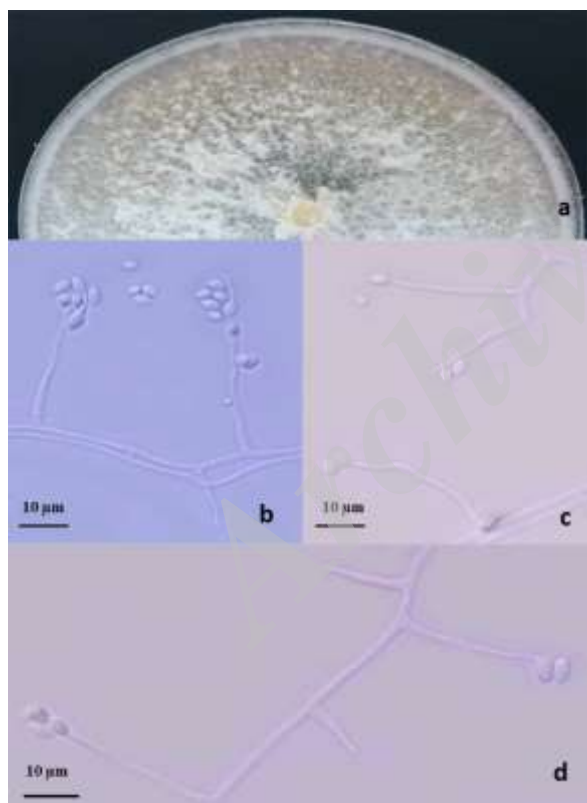


Fig. 2. *Emericellopsis pallida*. a. 10-d-old colony on oatmeal agar (OA); b-d. Branched conidiophores and conidia.

ACKNOWLEDGMENT

This work partially supported by an Ohio Agricultural Research and Development Center startup grant to Jason C. Slot. We acknowledge the Research Deputy of the University of Tabriz, Iran.

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گزارش دو گونه جدید از قارچ‌های هیفومیستی جداسازی شده از خاک‌های آلوده به نفت برای میکوبیوتای ایران

الهام محمدیان ✉، مهدی ارزنلو، اسداله بابای اهری

گروه گیاه پزشکی، دانشگاه تبریز، تبریز

چکیده: طی مطالعه تنوع قارچی خاک‌های آلوده به نفت استان خوزستان دو گونه جدید از هیفومیست‌ها برای میکوبیوتای ایران جداسازی شدند و بر اساس داده‌های ریخت‌شناختی و توالی ITS-rDNA به عنوان گونه‌های *Alternaria obovoidea* و *Emericellopsis pallida* شناسایی و توصیف شدند. این مطالعه اولین گزارش از جداسازی و شناسایی این دو گونه از خاک‌های آلوده به نفت در دنیا می‌باشند.

واژه‌های کلیدی: آلودگی نفتی، تنوع قارچی، ITS، گزارش جدید