Fomitiporia mediterranea, a new basidiomycete species for mycobiota of Iran

Received: 26.09.2018 / Accepted: 21.11.2018

Zahra Mirsoleymani: PhD Student, Department of Plant Protection, School of Agriculture, Shiraz University, Shiraz 7144165186, Iran

Reza Mostowfizadeh-Ghalamfarsa✉: Prof., Department of Plant Protection, School of Agriculture, Shiraz University, Shiraz 7144165186, Iran (rmostofi@shirazu.ac.ir)

During last decade, the decline of elm trees was observed in Fars province (Iran) due to a wood decaying agent in heartwood of the trees. Unknown basidiomycete isolates were frequently isolated from such trees. The objective of the present study was to identify the basidiomycete isolates by molecular phylogenetic analysis of the internal transcribed spacers of ribosomal DNA. For this purpose, isolates of a basidiomycete were recovered from infected elm trees with brown rot in heartwood in Fars province. The isolates developed buff-colored colonies with white margins which produced red-brownish pigments in both PDA and MEA media. Mycelial width was average 2.4–3.8 µm, with brown cell wall without clamp connections. No aerial mycelia were observed in any isolates and average growth rate of colonies was 1.35 mm^{-1} at 25 °C. No sexual organs were observed after six months of incubation at 25°C. Neighbor-joining phylogenetic analysis of internal transcribed spacers of rDNA (ITS) of sequences showed that the isolates belong to *Fomitiporia mediterranea*. This is the first report of *F. mediterranea* for Iran mycobiota. Small pieces of decaying wood from infected elm trees were placed on PDA and MEA at 25 °C and recovered isolates were purified on WA by hyphal tip method. Isolates were grown in 50 ml still culture of potato broth at 25 ºC. Freeze-dried mycelia were homogenized using sea sand (Fluka, Germany) and a plastic disposable pestle. Freeze-dried plant materials were also homogenized using mortars and pestles. DNA was extracted from homogenized preparation using a Genomic DNA Purification kit, (Fermentas, UK) according to the manufacturer's instructions. DNA of the internal transcribed spacer regions (ITS) were amplified using the universal primers ITS1:5'- TCC GTA GGT GAA CCT GCG G -3' and ITS4:5'- TCC TCC GCT TAT TGA TAT GC -3'. Amplifications were performed in a CG1-96 thermocycler (Korbett Research, Australia). The PCR mixture contained: 10–20 ng of template DNA, 1 µM of each primer, 100 µM of dNTPs, 0.4 U *Taq* DNA polymerase (CinnaGen, Iran), 1.5 mM of Mg Cl_2, 2.5 µl of 10× PCR buffer, 100 mM BSA, in a reaction volume of 25 µl. All PCRs consisted of 1 cycle of 94 °C for 3 min; 30 cycles of 95 °C for 30 s, 50 °C for 30 s, 72 °C for 60 s; and a final cycle of 72 °C for 10 min. PCR products were sequenced. Sequences of the internal transcribed spacer regions including the 5.8S gene of rDNA were used to study phylogenetic relationships of the studied taxa. The internal transcribed spacers sequences of rDNA generated in this study were compared to those of other taxa obtained from GenBank. A preliminary alignment of sequences was made using ClustalX with subsequent visual adjustment. Neighbor-joining phylogenetic analysis of internal transcribed spacers of rDNA (ITS) of sequences showed that the isolates belong to *Fomitiporia mediterranea* M. Fisch. (Fig. 1). The 780 bp sequence of isolate EN1 (GenBank Accession No.: HM582097) was 99% similar to ITS sequence from fruit body of *F. mediterranea* (Pilotti *et al.* 2005, GenBank Accession No.: AY620997) with some differences in 260 (T to C substitution) and 774 (A to gap substitution) nucleotide sites. *Fomitiporia mediterranea* was distinct by the sequences of the ribosomal DNA (ITS) region and growth rates at temperatures between 15 °C and 35 °C (Elena *et al*. 2006). The isolates developed buff colored colonies with white margins which produced red-brownish pigments in both PDA and MEA media (Fig. 2). Average mycelia width was 2.4–3.8 µm, with brown cell wall without clamp connections. No aerial mycelia were observed in any isolates and average growth rate of colonies was 1.35 mm d^{-1} at 25 °C. No sexual organs were observed after six months of incubation at 25 °C. The isolates were able to grow at all temperatures tested between 15 °C and 35 °C. This is the first report of *F. mediterranea* for Iran.

Specimen examined: Iran: Fars province, Shiraz, Bajgah, isolate EN1, recovered from elm trees, deposited at the Fungal Culture Collection of the Department of Plant Protection, Shiraz University, Shiraz, Iran (FT01.15.01).

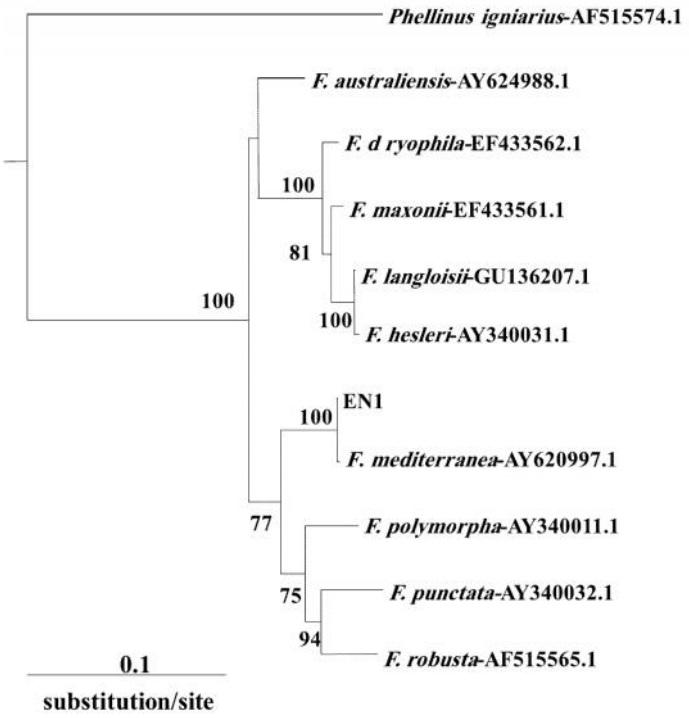

Fig. 1. Phylogram of a neighbour-joining analysis of the studied *Fomitiporia mediterranea* isolate EN1 (GenBank Accession No.: HM582097) together with different species of *Fomitiporia* based on ribosomal DNA (ITS) region. The numbers at the branch points indicate the percentages of bootstrap values ≥50%.

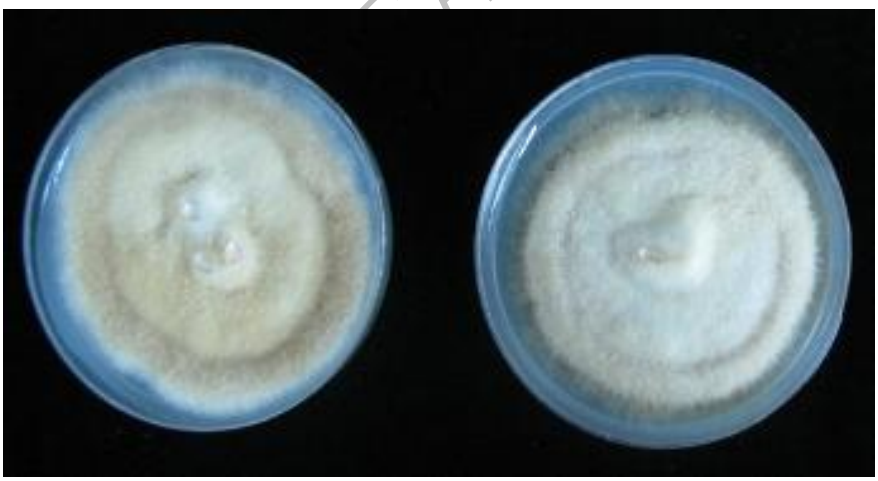

Fig. 2. Colony morphology of *Fomitiporia mediterranea* after 30 d at 25 °C on malt extract agar (MEA) (left) and potato-dextrose agar (PDA) (right).

References

Elena, K., Fischer, M., Dimou, D. & Dimou, D.M. 2006. *Fomitiporia mediterranea* infecting citrus trees in Greece. Phytopathologia Mediterranea 45: 35–39.

Pilotti, M., Gervasi, F. & Brunetti, A. 2005. Molecular identification of *Fomitiporia mediterranea* and *Eutypa lata/Libertella blepharis* in *Platanus acerifolia*. Journal of Phytopathology 153: 193–202.

Fomitiporia mediterranea، گونه‌ای جدید از بازیدیومیست برای میکوبیوتای ایران

دریافت: ۱۳۹۷/۰۷/۰۴ / پذیرش: ۱۳۹۷/۰۸/۳۰

زهرا میرسلیمانی: دانشجوی دکتری بیماری‌شناسی گیاهی، بخش گیاه‌پزشکی، دانشکده کشاورزی دانشگاه شیراز، شیراز، ۷۱۴۴۱۶۵۱۸۶ ایران

رضا مستوفی‌زاده قلمفرسا✉: استاد بیماری‌شناسی گیاهی، بخش گیاه‌پزشکی، دانشکده کشاورزی دانشگاه شیراز، شیراز، ۷۱۴۴۱۶۵۱۸۶ ایران (rmostofi@shirazu.ac.ir)

جدایه‌هایی از یک بازیدیومیست از نارون‌های آلوده به پوسیدگی قهوه‌ای عمق چوب در استان فارس به دست آمد. مشاهدات ریخت‌شناختی نشان داد که جدایه‌ها دارای پرگنه‌هایی با رنگ زرد و حاشیه سفید بوده و رنگدانه‌هایی با طیف رنگی قرمز-قهوه‌ای روی هر دو محیط کشت تولید کردند. میانگین عرض ریسه‌های قارچی ۳/۸-۲/۴ میکرومتر، دیواره سلولی قهوه‌ای رنگ و ریسه‌ها فاقد پل ارتباط بودند. ریسه‌های هوایی در هیچ یک از جدایه‌ها ملاحظه نگردید و میانگین رشد روزانه در ۲۵ درجه سلسیوس ۱/۳۵ میلی‌متر در روز بود. هیچ گونه اندام جنسی پس از شش ماه نگهداری در ۲۵ درجه سلسیوس مشاهده نشد. علاوه بر مشاهدات اولیه ریخت‌شناختی، پس از فزون‌سازی، خالص‌سازی و توالی‌سنجی جدایه‌های به دست آمده، واکاوی‌های فیلوژنتیک توالی‌های فاصله ترانویسی شده داخلی دی.اِن.اِی ریبوزومی (آی.تی.اِس) به روش پیوست همسایه‌ها نشان داد که جدایه‌ها مربوط به بازیدیومیست مولد پوسیدگی چوب *Fomitiporia mediterranea* هستند. این نخستین گزارش از وجود قارچ مذکور برای میکوبیوتای ایران است.

نمونه بررسی شده: استان فارس، شیراز، باجگاه، جدایه EN1 از *Fomitiporia mediterranea*، جدا شده از نارون، موجود در مجموعه قارچ‌های بخش گیاه‌پزشکی دانشکده کشاورزی دانشگاه شیراز (FT01.15.01).