Studies on root-endophytic *Monosporascus* from Europe

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Three novel fungal species belonging to the diatripaceous genus Monosporascus*:* M. bulgaricus*,* M. europaeus *and* M. solitarius*, were described as new root-endophytic fungi from Europe.*

Members of the fungal genus *Monosporascus* (Diatrypaceae, Xylariales) are characterized by globose or subglobose, perithecial or cleisthotecial ascomata containing one to six spherical, thick-walled, dark brown ascospores per ascus. Species such as *M. cannonballus* or *M. eutypoides* are important plant pathogens and the causal agents of root rot and vine decline of cucurbits worldwide, particularly melon and watermelon. However, recent culturable, phylogenetic and phylogenomic studies have revealed that its members are also ubiquitous root endophytes of different plant hosts and exhibit a remarkable diversity, with many *Monosporascus* isolates representing novel lineages still unknown to science and awaiting discovery [1].

During extensive sampling of root endophytic fungi across Europe, several *Monosporascus* isolates were obtained from surface-sterilized, asymptomatic roots of the brassicaceous host *Microthlaspi perfoliatum*. A first comparison of their nuclear ribosomal DNA internal transcribed spacer region (ITS) with sequences available in GenBank showed that these isolates were not conspecific with any previously described *Monosporascus* species. Therefore, they were plated on different culture media such as malt-extract agar (MEA) or potato dextrose agar (PDA) to induce sporulation and to further study their culturable features. None of them, however, sporulated on any of these media and they were considered sterile. Other markers used in phylogenetic studies of diatrypaceous fungi such as the gene encoding the β-tubulin protein or *tub2* were sequenced and used for analyses. Based on the integration of their phenotypic and molecular data, three novel species were characterized and newly described.

The first species was *M. bulgaricus*, isolated from roots of the plant host collected in Bulgaria [2]. Its colonies on MEA are fast growing, velvety, white but cottony, umbonate and raised 2–3 mm at the center, with flat, dull-white patches of scarce aerial mycelium all over the colonies or forming a concentric ring around the center, irregular margin and dull white reverse. Colonies on PDA are also velvety but somewhat cottony around the edges, dull white, mostly flat but slightly raised 1–2 mm at the center with a diffuse margin and similar reverse. In our ITS-LSU-*tub2* phylogeny, the two isolates of *M. bulgaricus* clustered together in a distinct and strongly supported lineage with a "Fungal sp. isolate 144" whose ITS sequence

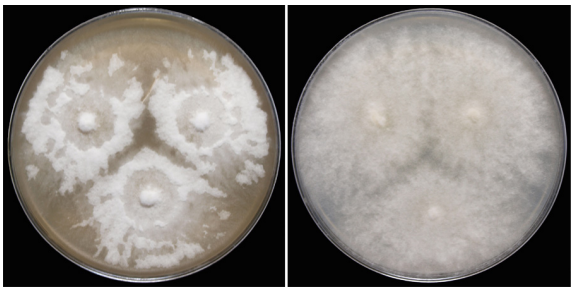

Figure 1: *Monosporascus bulgaricus*. Colonies on MEA and PDA after 1 week at 24 °C. Source: [2]

was available in GenBank. This is another root endophyte obtained from a grass species during studies of endophytic communities along altitudinal gradients in the Colorado Rocky Mountains, USA. Their ITS sequences, however, differ in several indels and this isolate probably represents another novel taxon within the apparent hyperdiverse *Monosporascus*. The three strains grouped sister to a large clade containing most described taxa including the type species, *M. cannonballus*, and several others such as *M. brasiliensis*, *M. caatinguensis* and *M. mossoroensis*. These are also root endophytes whose cultures were sterile on several different media and failed to produce the typical perithecial or cleistothecial ascomata of the genus.

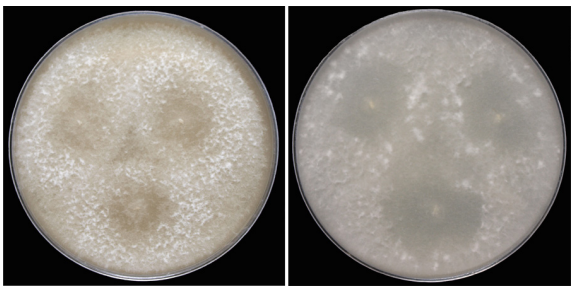

Figure 2: *Monosporascus europaeus*. Colonies on MEA and PDA after 1 week at 24 °C. Source: [3]

The second species was *M. europaeus*, isolated from roots of the host also obtained in Bulgaria [3]. Curiously, *M. europaeus* and *M. bulgaricus* were both isolated from plants of the same species collected at the same site on the same day, indicating that they coexist within the same host and location. Phylogenetically, however, they grouped distant and strains of *M. europaeus* formed a clade sister to several *Monospo-*

rascus species with no support, whereas *M. bulgaricus* represented an isolated lineage within the genus. Pairwise alignments of their individual ITS, LSU and *tub2* sequences also show several differences between them suggesting they are not conspecific. In culture, although both species were fast growing in all media after 1 week of incubation at 24 °C, colonies of *M. europaeus* on MEA are cottony, white and with scarce aerial mycelium around the center, whereas those of *M. bulgaricus* form a dull-white and flat concentric ring around the umbonate, cottony and white center. On PDA, on the other hand, colonies of *M. europaeus* are flat and circular around a cream-colored center, scarcely cottony and white toward the edges, whereas those of *M. bulgaricus* are velvety, somewhat cottony around the edges and dull white. Remarkably, the two strains of *M. europaeus* clustered together in a strongly supported monophyletic group (100% BS, 1 BPP) with six isolates represented in GenBank by their ITS sequences, implying a possible wider distribution of the fungus in disparate locations such as Michigan and New Mexico in the USA, or China and Iraq. Cultures of *M. europaeus* remained sterile in all media but formation of hyphal structures resembling ascomata initials made of tightly packed, coiled hyphae were observed after long-term incubation on MEA. However, further development into protoascomata was not obtained.

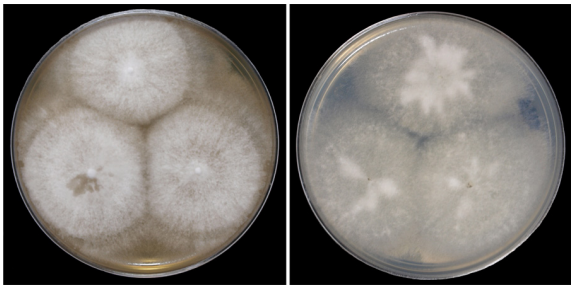

Figure 3: *Monosporascus solitarius*. Colonies on MEA and PDA after 1 week at 24 °C. Source: [4]

The third novel *Monosporascus* species was *M. solitarius*, isolated from roots of the same brassicaceous host in Greece [4]. A comparison with the two previously described taxa shows differences in culturable features. The three of them form white, fast-growing, sterile colonies on culture media but on MEA, for example, *M. bulgaricus* produces a dull-white and flat concentric ring around the umbonate, cottony center, whereas colonies of *M. europaeus* are cottony with scarce aerial mycelium around the center and those of *M. solitarius* are velvety, flat, and somewhat cottony toward the edges. Phylogenetically, they were all distantly related, and *M. solitarius* forms an isolated lineage sister to a group of strains named "*M. cannonballus*" without support. These strains are endophytes isolated from tissues of the grass *Stipagrostis ciliata* and distant from ATCC 26931, the ex-type strain of *M. cannonballus*, probably representing an undescribed species. Moreover, unlike *M. bulgaricus* or *M. europaeus*, megablast searches of the ITS region of *M. solitarius* in GenBank did not retrieve any closely related strains from previous ecological or phylogenetic studies.

The recent discovery and description of these novel root-endophytic species from Europe further supports the putative hyperdiversity of *Monosporascus*, and suggests that many more additional taxa likely remain undiscovered within the genus.

Notes

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References

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