

Morphological and preliminary molecular characterizations of low temperature *Pythium* from grasslands in East-Azarbaijan province, Iran

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The Genus *Pythium* contains more than 140 recognized species and is distributed worldwide in diverse environments including the cryosphere (Tojo and Newsham 2012, Kageyama 2014). Some species of low temperature *Pythium* cause severer snow rot on golf course turfgrass (Masumoto et al. 2010) and natural vegetation (Tojo et al. 2012). Snow rot has commonly been found in agricultural and natural grasslands in East-Azarbaijan province (Fig. 6), Iran (Chenari Bouket and Arzanlou, unpublished). We recently isolated several species of low temperature *Pythium* from grasses with snow rot symptoms (Fig. 1). Present study reports their morphology and primary molecular examination as the first step to clarify their taxonomical status.

Materials and Methods

Isolation. – All isolates were recovered from snow covered grasslands in East-Azarbaijan province, Iran. Pieces of diseased grass leaves (*Lolium perenne*) were plated in NARM (Morita and Tojo 2007) and VP3 (Ali-Shtayeh et al. 1986), oomycete-specific media and incubated at 4°C for 3 weeks. After purification, cultures were maintained in PCA slant vials at 4°C. Morphological observations were made on structures produced on PCA as well as those on sterile grass blades floated in sterile pond water.

DNA extraction, PCR and Electrophoresis. – DNA was extracted from mycelia grown on PDA (Moller et al. 1992). The ITS region of rDNA was amplified by polymerase chain reaction using primers ITS1 and ITS4 (White et al. 1990). Amplification was carried out in a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA) according to the following amplification program: 5 min at 96 °C for primary denaturation, followed by 40 cycles of 94 °C for 30 s, 52 °C for 30 s, 72 °C for 60 s, with a final extension at 72 °C for 7 min. PCR products were examined in 1.2% agarose Gel, at ~ 100V for 1h with 1Kb DNA Ladder (BioTools).

Results and Discussion

Preliminary molecular evaluation and morphological observations showed that at least three species of *Pythium* were present in rotted grass leaves under the snow in East-Azarbaijan province (Fig. 2 and Fig. 3 to 5). They were tentatively identified as *Pythium* spp. 1, 2, and 3. *Pythium* sp. 1 was characterized by filamentous, spherical and intercalary sporangia. Subspherical sporangia produce apical papilla (Fig. 3). *Pythium* sp. 2 was characterized by spherical sporangia, oogonium with one and two antheridia and subspherical, filamentous structures that resembled appresoria. Sporangia germinated through papillae (Fig. 4). *Pythium* sp. 3 was characterized by subspherical and spherical sporangia, oogonium with one and two antheridia and plerotic oospores (Fig. 5).

These results demonstrated that low temperature *Pythium* were present in East-Azarbaijan province, Iran. This is the first report of low temperature *Pythium* in Iran. Their phylogenetic characterization is under way.

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