First Report of *Rhizopus oryzae* Causing Potato Soft Rot in the Hebei Province of China

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Rhizopus soft rot occurs on the succulent tissues of vegetables, fruits and ornamental plants throughout the world. In September 2016, a disease outbreak in potato suspected as *Rhizopus* soft rot occurred in two fields in Guyuan County, Zhangjiakou City, Hebei Province of China. There were soft water-soaked lesions on the surface of diseased tubers, and the interiors were black and soft. Infected plants were sampled to isolate and determine the identity of the disease-causing organism. Symptomatic tubers were surface-sterilized with 1% NaClO for 1 min, rinsed three times with autoclaved distilled water. Small pieces of the inner decayed tissue were removed and cultured on potato dextrose agar (PDA) at 28°C for 2-3 days. Fifteen white colonies

grew rapidly on PDA, they became brownish-grey to blackish-grey mycelium. One colony was isolated (MLS-22-1-z15) for identification. Under the microscope, non-septate rhizoids, sporangia and sporangiospores were observed. Sporangiospores were unequal, numerous irregular, sub-globose or oval, and 5.7 (3.5 to 8.2) × 4.7 (3.1 to 6.5) µm (n = 50) in diameter. The sporangia were globose, black, 120.2 (64.5 to 165) µm (n = 50) in diameter. We analyzed the sequence of the internal transcribed spacer (ITS) region between genes coding for the large and small rRNA genes to determine the identity of the MLS-22-1-z15 isolate. The sequence of ITS was determined by PCR and Sanger sequencing using ITS4/ITS5 primers (Abe et al. 2010). BLAST analysis of the MLS-22-1-z15 isolate ITS sequence (accession no. MH046841) revealed 100% sequence identity with *Rhizopus oryzae* (accession no. KU203330.1). Based on the morphological characteristics (Zhang et al. 2013; Suyala et al. 2008) and rDNA-ITS sequencing, the MLS-22-1-z15 isolate was identified as *R. oryzae.* To test whether we had isolated the disease-causing organism, we inoculated healthy tubers with isolate MLS-22-1-z15 by using a wound inoculation method (Choiseul et al. 2007) and observed disease development and progression. The disease-free potato tubers were rinsed three times with autoclaved distilled water, and surface sterilized with 75% ethanol. A 3 mm plug was removed from the surface sterilized tubers using a cork borer, and a 3-mm-diameter agar plug from the edge of a 2-day-old colony was placed in the hole. An uninoculated agar plug was inserted into the wound as a negative control. These results were replicated in three independent trials, with each including three tubers, for both treatments. After incubation at 28°C for 3 days, all of the inoculated tubers had extensive decay and gave off a foul-smelling odor. The control tubers had no obvious symptoms. *R. oryzae* was re-isolated from the symptomatic potato tubers and confirmed as such based on colony and sporangia morphology and ITS sequence. To our knowledge, this is the first report of *R. oryzae* associated with soft rot on potatoes in China.

References

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