Disease Notes

First Report of *Penicillium polonicum* Causing Bl Mold on Stored Onion (*Allium cepa*) in Serbia

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Abstract

Penicillium polonicum K. Zaleski is an economically important airborne fungus with a broad host range including cereals, peanuts, onions, dried meats, citrus fruits, and yam tubers (2,4). Secondary metabolites produced by this species include harmful mycotoxins penicillic acid, verucosidin, and nephrotoxic glycopeptides, which may play a role in Balkan Endemic Nephropathy (2,5). In January 2013, decayed onion bulbs (*Allium cepa* L. cv. Meranto) with blue mold symptoms were found causing significant economic losses at a storage facility in Stara Pazova, Serbia, and were collected. The decayed area of the bulbs was pale yellow to light brown, and tissue was soft and watery. Bluish green sporulation was abundant on the surface and inside the bulb, between decayed scales. Two isolates (designated L1a and L4p) were obtained and further characterized using morphological and molecular methods. Colonies on potato dextrose agar (PDA), Czapek yeast autolysate agar (CYA), malt extract agar (MEA), and yeast extract sucrose agar (YES) media at 25°C after 7 days

were blue green, velutinous, with clear exudate present on CYA. Colony reverse color on CYA and YES for both isolates were cream to yellow brown. The mean colony diameter on PDA for L1a was 29.89 ± 0.96 mm, and for L4p was 26 ± 0.37 mm; on CYA 32.56 ± 0.53 mm for L1a and 30.11 ± 2.42 mm for L4p; and on YES 33.86 ± 1.59 mm for L1a and 31.17 ± 1.83 mm for L4p. No growth was observed on CYA when isolates were incubated at 37°C. Conidiophores of both isolates were terverticillate; stipes were septate with smooth to finely roughened walls, and phialides were ampulliform. Conidia were globose to subglobose, smooth-walled, and borne in columns. Conidial dimensions for L4p were 2.72 to 3.82 (3.26) × 2.36 to 3.42 (2.95) µm, and for L1a were 2.87 to 4.39 (3.58) × 2.53 to 3.79 (3.16) µm (*n* = 50). Both isolates tested positive for the production of cyclopiazonic acid and other alkaloids, as indicated by a violet reaction for the Ehrlich test. Morphological characters of L1a and L4p were in accordance with those described for *P. polonicum* K. Zaleski (2). Genomic DNA was isolated using CTAB extraction method (1) and molecular identification was completed using gene specific primers for the β-tubulin locus (Bt-LEV-Up4/Bt-LEV-Lo1) via conventional PCR (3). The nucleotide sequences of amplified products (~800 bp) have been assigned to GenBank (KJ570971 and 72). MegaBLAST of obtained sequences showed a 99% similarity with several sequences of *P. polonicum* deposited in GenBank, which confirmed the morphological identification. Pathogenicity was tested by wound inoculation of 10 surface sanitized onion bulbs cv. Meranto with 50 µl of a 10^5/ml conidial suspension from isolates grown on PDA. Ten control onion bulbs were wound-inoculated with Tween-treated sterile distilled water. After 30 days incubation in plastic containers, under high humidity at 22°C, typical symptoms of blue mold developed on inoculated bulbs, while non-inoculated controls remained symptomless. Isolates recovered from inoculated bulbs showed the same morphological characteristics as the original isolates, thus completing Koch's postulates. To our knowledge, this is the first report of *P. polonicum* on stored onion in Serbia. Results from this study indicate that a holistic approach to control this fungus should be implemented that may include one or all of the following: increased sanitation methods to eliminate inoculum, breeding for resistant onion cultivars, and integration of additional control methods to maintain onion quality during storage.

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