Disease Notes

First Report of *Penicillium crustosum* Causing Blue Mold on Stored Apple Fruit in Serbia

I. Vico, V. Gaskins, N. Duduk, Miljan Vasić, J. J. Yu, K. A. Peter, and W. M. Jurick II

Affiliations

Authors and Affiliations

I. Vico , University of Belgrade, Faculty of Agriculture, Institute of Phytomedicine, Plant Pathology Department, Nemanjina 6, 11080 Belgrade, Serbia
V. Gaskins , USDA-ARS, Food Quality Laboratory, Beltsville, MD 20705
N. Duduk
Miljan Vasić , University of Belgrade, Faculty of Agriculture, Institute of Phytomedicine, Plant Pathology Department, Nemanjina 6, 11080 Belgrade, Serbia
J. J. Yu , USDA-ARS, Food Quality Laboratory, Beltsville, MD 20705
K. A. Peter , Penn State University, Department of Plant Pathology and Environmental Microbiology, Fruit Research and Extension Center, Bigerlville, PA, 17307
W. M. Jurick II , USDA-ARS, Food Quality Laboratory, Beltsville, MD 20705

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Abstract

Penicillium crustosum Thom (1930) causes blue mold on pome fruits and is also regularly found on cheese, nuts, and soil (1,3). The fungus produces a wide range of mycotoxins such as penitrem A, roquefortine C, terrestric acid, and cyclopenol, which impact human health (1). In January and February 2013, 20 decayed apples, 'Golden Delicious' and 'Jonagold' (*Malus* × *domestica* Borkh.) with blue mold symptoms were collected from cold storages in Svilajnac and Bela Crkva, Serbia. Decayed areas were light to medium brown with blue green sporulation on the surface of the lesion. Decayed tissue was soft and watery with a sharp margin between the diseased and healthy areas. One isolate from each cultivar was designated JP2 ('Golden Delicious') and JBC7 ('Jonagold') and further characterized. Conidiophores of both isolates were

terverticillate, stipes were septate with rough walls, and phialides were ampulliform. Conidia were smooth, borne in columns, and were spherical to subglobose. Conidial dimensions for JP2 were 3.2 to 4.56 (3.73) × 2.64 to 4.3 (3.32) µm and for JBC7 were 3.1 to 4.46 (3.65) × 2.81 to 4.27 (3.31) µm (*n* = 50). The isolates were cultured on Czapek yeast autolysate agar (CYA), malt extract agar (MEA), and yeast extract sucrose agar (YES) media and incubated at 25°C for 7 days. Mycelia were white w... heavy sporulation yielding grayish green colonies on all media. Colonies were ra... sulcate and velutinous, with clear exudate, and produced a yellow to orange reverse on CYA and YES. On MEA, colonies were plane, low, and mycelia subsurface with conidia having a dry powdery appearance. Crusts of conidial masses formed after 10 or more days. No growth was observed on CYA when these isolates were incubated at 37°C. Both isolates were identified as *P. crustosum* Thom using morphological characters according to (2) and (1). Species level identification was confirmed by isolating genomic DNA followed by amplification of the β-tubulin locus using gene specific primers via conventional PCR (4). MegaBLAST analysis of the 2X consensus nucleotide sequences revealed that JP2 and JBC7 (GenBank KJ433984 and 85) were 99% identical to *P. crustosum* culture collection isolate IBT 21518 (JN112030.1). Koch's postulates were examined using two apple cvs. Idared and Kolacara. Ten fruit per cultivar per isolate were inoculated on two sides of each fruit; 20 fruit were used as water-only inoculated controls. Fruit were washed with soap and water, surface sanitized with 70% ethanol, and placed into polyethylene boxes. Using a finishing nail, 4-mm wounds were created and inoculated with 50 µl of a 3×10^5/ml conidial suspension or Tween-treated sterile distilled water. Boxes with inoculated and control fruit were stored at 25°C for 10 days. The inoculated fruit developed small, soft, watery lesions, which enlarged into decayed areas with defined edges and abundant sporulation on the surface. Symptoms were identical to the original ones, while the control fruit remained symptomless. The fungus was re-isolated from infected tissue and showed the same morphological characteristics as the original isolates, thus completing Koch's postulates. Blue mold occurs during long term storage of apples and is predominantly caused by *P. expansum.* This is the first report of *P. crustosum* causing postharvest blue mold decay on apple fruit obtained from storage in Serbia and indicates that *P. crustosum* is an emerging pathogen for the Serbian pome fruit growing and packing industry.

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3340 Pilot Knob Road, St. Paul, MN 55121

USA

+1.651.454.7250

FAX +1.651.454.0766

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