Diseases Caused by Bacteria and Phytoplasmas

First Report of Brown Spot on White Button Mushroom (*Agaricus bisporus*) Caused by *Cedecea neteri* in Iran

Mozhde Hamidzade,¹ S. Mohsen Taghavi,¹ Hamzeh Mafakheri,¹ Rachel A Herschlag,² Samuel J. Martins,² Kevin L. Hockett,² Carolee T. Bull,² and Ebrahim Osdaghi^{3,†}

¹Department of Plant Protection, School of Agriculture, Shiraz University, Shiraz 71441-65186, Iran

²Plant Pathology & Environmental Microbiology Department, The Pennsylvania State University, University Park, PA 16802, U.S.A.

³Department of Plant Protection, College of Agriculture, University of Tehran, Karaj 31587-77871, Iran

Current address for S. J. Martins: Department of Plant Pathology, University of Florida, Gainesville, FL 32611, U.S.A.

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In autumn 2018, cap discoloration and browning symptoms (up to 20% incidence) were observed on commercially grown white button mushrooms (*Agaricus bisporus*) in two distinct farms located in Shiraz and Marvdasht Counties in Southern Iran. Symptomatic caps (13 and 6 caps from Shiraz and Marvdasht Counties, respectively) were characterized by visible brown discoloration with no blotch symptoms, bacterial sticky mass, and cap wilting. Isolation of bacterial strains from infected cap tissues was performed on yeast-extract peptone glucose agar according to Hamidzade et al. (2020). The resulting bacterial colonies were oyster-white in color, non-fluorescent, domed convex circular, with smooth margins 1 to 2 mm in diameter. In total, six bacterial strains (Shiraz: Ir1002, Ir1003, Ir1004, Ir1005, and Ir1007 and Marvdasht: Ir1027) were isolated from distinct mushroom samples. Standard biochemical and phenotypic analyses (Schaad et al. 2001) showed that the bacterial strains were Gram negative and oxidase negative, catalase positive, and facultatively anaerobic, while no capsule or endospore was observed. All strains were positive in urease production, arginine dihydrolase, hydrolysis of Tween 80, and utilization of sucrose and D-sorbitol, while they were negative in amylase, cellulose, lecithinase, pectinase, and protease production as well as casein hydrolysis.

Based on these phenotypic characteristics, the strains were supposed to be members of the family *Enterobacteriaceae*. They also did not induce hypersensitive reaction on tobacco (*Nicotiana tabacum* 'Turkish') leaves, nor did they produce tolaasin when streaked side-by-side with *Pseudomonas reactans* strains on King B medium (Osdaghi et al. 2019). Pathogenicity of the strains was evaluated (repeated twice) on fresh caps of white button mushroom using the cap-cutting method (Hamidzade et al. 2020). Reference strains of *P. tolaasii* (CFBP 8707) and *Mycetocola* spp. (CFBP 8708) were used as positive controls, while sterile distilled water was used as a negative control. Brown discoloration appeared 24 to 36 h postinoculation on cap surfaces while control caps remained asymptomatic. Koch's postulates were accomplished by reisolation and identification of bacterial strains from the symptomatic caps using colony morphology and Gram staining. For molecular identification, all initial as well as reisolated strains were subjected to amplification and sequencing of 16S rDNA and *gyrB* (Hamidzade et al. 2020; Yamamoto and Harayama 1995). Nucleotide sequences obtained were deposited into NCBI GenBank (16S: MZ298620 to MZ298625; *gyrB*: MZ313184 to MZ313189). BLAST search using the 16S rDNA and *gyrB* sequences showed that the strains isolated in this study had 97 to 99% sequence similarity to the reference strains of *Cedecea neteri*. Phylogenetic analyses also confirmed a close relationship of bacterial strains from this study to *C. neteri* strains. Pure cultures of representative strains Ir1004 (CFBP 8900) and Ir1027 (CFBP 8896) are deposited in the CIRM-CFBP culture collection. This is the first report of *C. neteri* causing brown spot disease on button mushroom in Iran, while the bacterium has previously been reported to cause soft rot on *Pholiota nameko* (Yan et al. 2018) and yellow sticky disease on *Flammulina velutipes* (Yan et al. 2019) in China. Further comprehensive investigations will shed a light on the economic impact of brown spot disease on the mushroom industry in Iran.

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[†]Indicates the corresponding author.
E. Osdaghi; eosdaghi@ut.ac.ir