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Clinical Image

First Record of *Geotrichum candidum* Causing Root Rot to Sugar beet (*Beta vulgaris*. L) in Wyoming and Michigan, USA

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Clinical Image

Sugar beet (*Beta vulgaris* L.) root rotten is caused by a number of soil-borne pathogens included *Rhizoctonia solani*, *Aphanomyces cochlioides*, *Clonostachys rosea*, *Globisporangium ultimum*, *Rhizopus stolonifera*, and *Fusarium equiseti* [1-4]. In September 2019, water-soaked soft rot with brown irregular lesions with a fermented smell, and thick foamy white mycelial growth symptoms were observed on sugar beet roots collected from Wyoming and Michigan (Figure 1 and 2). The symptoms covered approximately 40% of the sugar beet root surface. Diseased beet root tissues were excised from the junction of diseased and healthy tissue. Small pieces (10mm^2) were surface sterilized with 10% sodium hypochlorite for 1min, rinsed thrice with sterile distilled water, air-dried on laminar airflow cabinet for 5 minutes, and transferred to corn meal agar (CMA), and clarified V8 (CV8)- incubated at 25°C with a 12-h photoperiod for 7 days. White, thin, flatted, and feathery colonies appeared on both media (Figure 3 and 4). Conidia were arthrosporous, mostly like chains, segmented, hyaline, subglobose, and single-celled (Figure 5). The dimension of conidia varied from 5-12 x 2-5 µm [5]. Based on microscopic and macroscopic characters, the fungus was speculated to be Geotrichum species. Ten isolates were developed by the single spore isolation technique. To confirm the species of the fungus, Genomic DNA was extracted from two representatives of the isolates and the internal transcribed spacer (ITS) region was amplified using the ITS1/ITS4 primers [6]. The amplified PCR products were cleaned and sent for Sanger sequencing by GenScript (GenScript, Piscataway, NJ). A Blastn search of the ITS sequences from the isolates showed 100% identity to those of *Geotrichum candidum* isolates KF713519.1 and MF782775.1. Pathogenicity assay was conducted by wounding the sugar beet surface with a sterile scalpel after scrapping 7-days old CMA culture and kept in the cold room for 10 days. Six sugar beetroots used as biological replicates with two replications were used for the study (Figure 6). Likewise, mock-inoculated sugar beet

Figure 1: Infected beet collected from sugar beet field in Wyoming.

Figure 2: Infected beet collected from sugar beet field in Michigan.

Figure 3: Appearance of *Geotrichum candidum* grown on corn meal agar (CMA).

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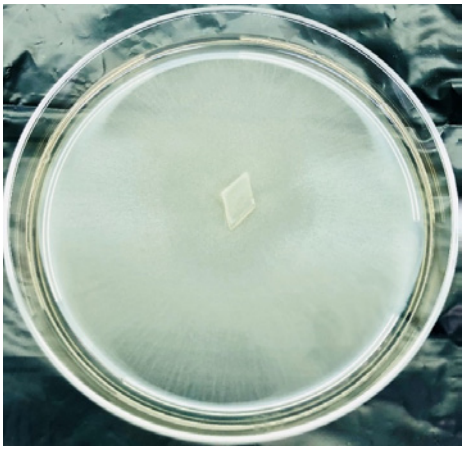

Figure 4: Appearance of *Geotrichum candidum* grown on clarified V8 agar (CV8) media.

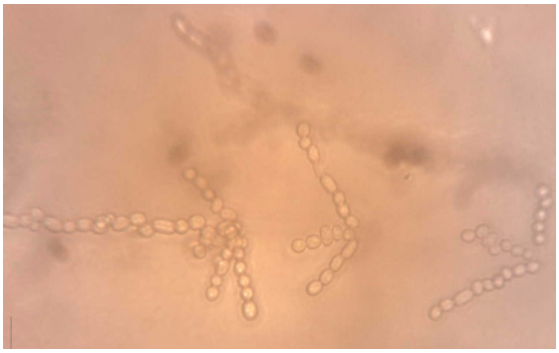

Figure 5: Morphological characteristics of *Geotrichum candidum*.

Figure 6: Symptoms of sugar beet inoculated artificially with *Geotrichum candidum*.

roots were also kept as a control. Two weeks of post-inoculation, all inoculated sugar beet roots were observed with the similar water-soaked soft rot with brown irregular lesions with a fermented smell and thick foamy white mycelial growth on the sugar beet. No symptoms were observed in the mock treatment. The experiment was conducted twice. The fungus was re-isolated from the diseased beet root tissue, as described above. Macroscopic and microscopic

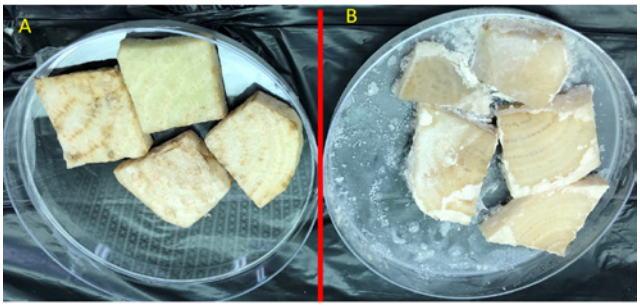

Figure 7: *In-vitro* inoculation of *Geotrichum candidum* on sugar beet slices A. non-inoculated check-sugar beet slice shows zero rotten, B. inoculated slices shows rotten signs and growth of white mycelia with fermented smell at 3 weeks after post-inoculation.

analysis indicated the re-isolated fungus had a similar whitish thin colony, and conidial structure, respectively, as the one originally used for the inoculation. Genomic DNA was extracted from two isolates as described above. Molecular analysis performed using the same ITS primers further confirmed that these isolates were *Geotrichum candidum*. In addition, *in-vitro* inoculation with conidial suspension of *Geotrichum candidum* on fresh sugar beet tissue slices was performed to understand the pathogenesis of *Geotrichum candidum* at room temperature. This shows infection like tissue rotten, foamy mycelial growth, and fermented smell in the inoculated sugar beet slices. The infection resulted in the discoloration of tissue which is followed by decomposition (Figure 7B). There was no tissue decomposition in the control plate (Figure 7A). This pathogen has recently been reported to cause sour rot of peach, lime, strawberry, mori, loquat, and sweet potato [7-11]. To our best knowledge, this is the first report of *Geotrichum candidum* causing disease in sugar beet root in Wyoming and Michigan. Notable, this pathogen was recently observed to be ubiquitous in major sugar beet production areas in North Dakota, Minnesota, Nebraska, and Colorado, therefore, proper management practices need to develop to save the sugar beet industry.

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