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Short Notes

Identification of *Plectosphaerella melonis* from cucumber plants in Ukraine

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Summary. A fungus was isolated from diseased roots of *Cucumis sativus* grown in greenhouses. The morphological and cultural characteristics of the isolate allowed it to be classified as *Plectosphaerella melonis*. BLASTn analysis revealed 99% homology of the ITS sequence from the isolate with 14 *Acremonium cucurbitacearum* and *P. melonis* isolates, allowing attribution of the isolate to *P. melonis* (syn. *A. cucurbitacearum*). Koch's hypothesis requirements were fulfilled for the isolate. Symptoms on host roots developed after 14 d of growing cucumber plants on infested soil. Plants of the cucumber variety Nizhynskyi 12 were very susceptible at the two leaf growth stage (2 weeks after sowing). Above-ground disease symptoms were absent after 14 d, even with severely diseased roots. This is the first report of *P. melonis* on *C. sativus* in Ukraine.

Keywords. *Acremonium cucurbitacearum*, cucurbits, disease, identification, pathogen, *Plectosphaerella melonis*, soilborne.

INTRODUCTION

Plectosphaerella spp. are known pathogens for a wide range of agricultural crops, including *Cucurbitaceae* (Carlucci *et al.*, 2012; Raimondo and Carlucci, 2018a; 2018b).

In Spain, Alfaro-García *et al.* (1996) reported that the causal agent of this disease, after the mass death of melons at the beginning of fruit ripening, was the fungus *Acremonium cucurbitacearum*. Bruton *et al.* (1996) described the same disease on melon plants in Texas (United States of America). Carlucci *et al.* (2012) determined that *A. cucurbitacearum* was synonymous to *Nodulisporium melonis* and transferred the pathogen to *Plectosphaerella* as *P. melonis* comb. nov.

A *Plectosphaerella* species was consistently isolated from diseased cucumber plants grown in a greenhouse in Ukraine. The aim of the present study was to identify the isolate fungus using morphological cultural and molecular genetic characteristics, and to confirm its pathogenicity to *Cucumis sativus*.

MATERIALS AND METHODS

Plant sampling and fungus isolation

A fungus isolate (coded 502) was collected from affected roots of *C. sativus* (variety Koroliok), at the mass fruiting stage of crop growth. *Cucumis sativus* was grown in greenhouses in Chernihiv district, Chernihiv region (Ukraine). Affected host root segments (3–5 mm) were thoroughly washed for 15 min under running water, surface sterilized with 96% ethyl alcohol for 1 min then washed twice with sterile water, and were then plated into Petri dishes containing 4% barley meal agar (BMA) amended with 250 ppm streptomycin. The plates were incubated at 26°C, and after 4 d the resulting fungus was reisolated onto BMA.

Morphological identification

The cultural and morphological characteristics of the isolated fungus were examined after culture on BMA, Czapek's agar (CZA) or potato dextrose agar (PDA) in the darkness at 26°C. After 10 d in culture, the fungus completely covered the agar surfaces, and colonies were characterised.

Temperature/growth relationship

The temperature optimum for the isolated fungus was studied by culturing the fungus in Petri dishes on BMA at 10, 18, 26 or 35°C. After 10 d, the colony diameters were measured in two mutually perpendicular directions, and colony radial growth rates were determined (Pert, 1978).

Molecular identification

DNA extraction. For DNA isolation, parts of fungus colonies grown on BMA were processed using the AmpliSens DNA-sorb-B kit. The necessary quantity of DNA solution obtainment was carried out in accordance to described methods (Birnboim & Doly, 1979; Chowdhury & Akaike, 2005; Chi *et al.*, 2009; Sika *et al.*, 2015).

PCR analysis. For sequencing, obtained DNA solution polymerase chain reaction was carried out using ITS1 and ITS4 primers (White *et al.*, 1990). The amplification reaction was conducted within Applied Biosystems equipment following prescribed methods (Watts & MacBeath, 2001; Garrido *et al.*, 2009). Analysis of resulting 5.8S rDNA sequences were compared with GenBank

database sequences using by BLAST analyses (<http://www.ncbi.nlm.nih.gov/blast>).

Pathogenicity test

Pathogenicity tests were performed in a pot experiment using *C. sativus* plants (var. Nizhynskiyi 12). Pots (2 L capacity) were each filled with 1800 g of soil which had been previously steamed at 70–80°C for 50 min.

Inoculum of the isolated fungus was prepared by growing the fungus on medium containing oat seeds, oat flakes, water, chalk and gypsum in 1 L capacity flasks. This substrate was sterilized twice at 128°C and 1.5 atm pressure for 1 h 30 min, and was then inoculated with pure cultures of the isolate, grown in tubes containing BMA. The flasks were kept at 26°C for 21 d. Then the inoculum was transferred into paper bags, dried to constant weight at 30 °C, and mixed with soil.

Cucumis sativus seeds were sown to a depth of 2 cm. Fungus inoculum was applied to the pots at the equivalent of 5×10^4 CFU g⁻¹ of soil (Bruton *et al.*, 2000a). Experimental controls were cucumber plants grown without added inoculum. Soil in the pots was maintained at 60% of total water holding capacity. The pots were placed randomly in a greenhouse with natural lighting. The experiment was repeated five times. After emergence of the cucumber seedlings, these were thinned to five per pot. After 2 and 4 weeks, the plants were removed, washed from the soil and the root systems were evaluated.

Root segments (3–5 mm each) were thoroughly washed for 15 min under running water, surface sterilized with 96% ethyl alcohol for 1 min, and then washed twice with sterile water. The segments were then placed into Petri dishes containing 4% BMA plus 250 ppm Streptomycin at 26°C, and after 4 d the resulting fungus was reisolated onto BMA.

Statistical analyses

The data obtained were analyzed using Statistica 12. Normality of the data were assessed using Shapiro-Wilks W-test statistics, and homogeneity of variances was assessed using the Brown-Forsythe test. The nonparametric Mann-Whitney *U* test was applied ($P \leq 0.05$) to compare experimental groups, including fungus growth media. One-way Analysis of Variance (ANOVA) and Duncan Multiple Range Tests were used to determine optimum temperature.

RESULTS AND DISCUSSION

Isolate 502 was obtained from affected cucumber plants grown in greenhouses (Figure 1a). Disease symptoms were absent until the onset of the mass plant fruiting phase of cucumber. The symptoms included fading of leaf edges on the lower leaves of affected plants. The root systems had secondary and tertiary roots with necrotic areas (Figure 1b). No reductions in plant growth or delays in development were observed.

The macro-morphological features of isolate 502 differed depending on the culture medium (Figure 2). Colonies were rounded, hyphae were thin, septate and hyaline. The colonies on PDA were white or cream, the colony surfaces were and velvety. On BMA, the colony colours varied from white to rose pink. On CZA the colonies had white aerial mycelium. Colonies had radial wrinkles on the reverse sides, and the colonies were

slow-growing (Table 1), the most rapid growth was on PDA.

Phialides of isolate 502 were unbranched, septate, mostly simple, and measured from $22.4 \times 4.2 \mu\text{m}$ to $42.0 \times 4.2 \mu\text{m}$. The phialides were coloured along their entire lengths. The phialides were located on hyphal filaments and each had a basal septum at the base. Unicellular conidia were assembled into a head at the apex of each phialide. Conidia were oblong, elliptical, smooth-walled and hyaline (granular). Conidia in PDA cultured measured $8.4 \times 2.8 \mu\text{m}$ to $14.0 \times 2.8 \mu\text{m}$, and sporulation was most intense on this medium. On BMA, the conidia were smaller ($5.6 \times 3.1 \mu\text{m}$ to $11.2 \times 2.8 \mu\text{m}$) and sporulation was intense. On CZA, sporulation was not intense and the conidia were $4.2 \times 4.2 \mu\text{m}$ to $8.4 \times 4.2 \mu\text{m}$. Chlamydo spores were absent. These characteristics were similar to those described for *P. melonis* (Alfaro-Garcia *et al.*, 1996; Carlucci *et al.*, 2012).

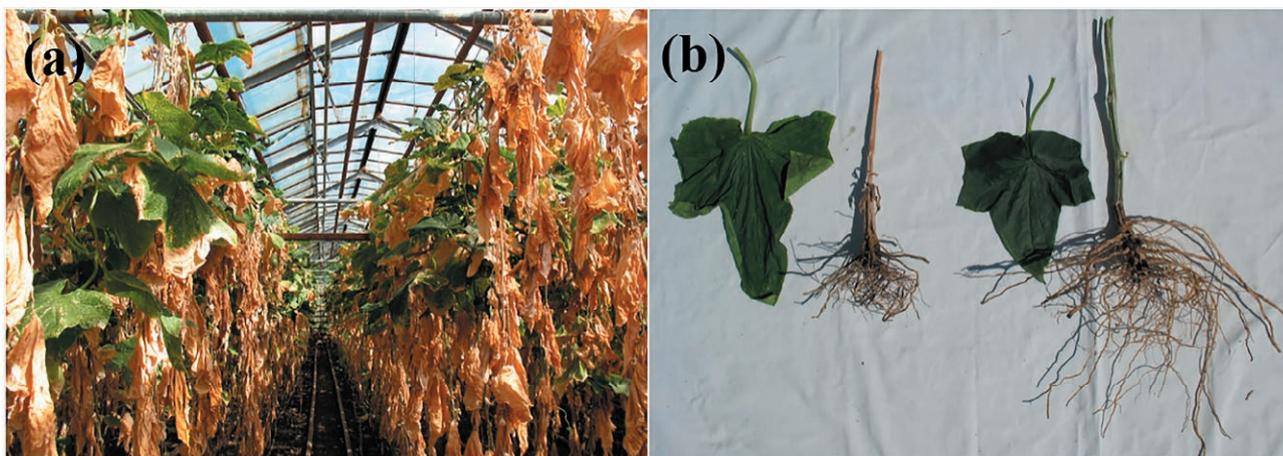


Figure 1. (a) Cucumber plants (variety Koroliok) affected by *Plectosphaerella melonis* (isolate 502) in a greenhouse. (b) Leaf and root of an affected plant (left) and a healthy plant (right).

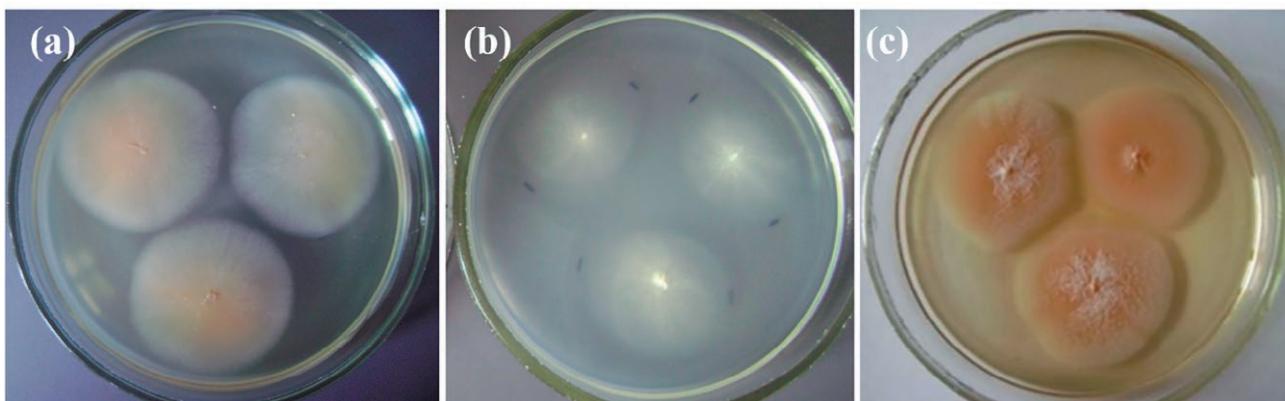


Figure 2. Colonies of *Plectosphaerella melonis* (isolate 502) on (a) PDA, (b) CZA, and (c) BMA.

Table 1. Mean diameters of *Plectosphaerella melonis* (isolate 502) colonies on different nutrient media.

Nutrient medium	Mean colony diameter (mm) after 7 d.	Mean colony diameter (mm) after 10 d.
BMA	20.2 ± 0.2 c	26.8 ± 0.1 c
CZA	17.1 ± 0.1 b	28.5 ± 0.3 b
PDA	21.2 ± 0.3 a	33.0 ± 0.3 a

The values are means ± standard errors for three replicates. Means in each column followed by different letters are significantly different ($P \leq 0.05$; Mann–Whitney test).

Table 2. Mean *Plectosphaerella melonis* (isolate 502) colony diameters and growth rates at different temperatures.

Temperature (°C)	Mean colony diameter (mm, after 10 d)	Mean colony radial growth rate (mm h ⁻¹)
10	5.5 ± 0.3 d	0.023 ± 0.001 d
18	13.2 ± 0.5 b	0.055 ± 0.002 b
26	22.3 ± 0.3 a	0.093 ± 0.001 a
35	8.8 ± 0.3 c	0.037 ± 0.001 c

The values are means ± standard errors for three replicates. Means in each column followed by different letters are significantly different ($P \leq 0.05$; Duncan's multiple range test).

The optimum temperature growth for isolate 502 was 26°C (Table 2).

Isolate 502 was deposited at the Depository of the Institute of Microbiology and Virology, NAS of Ukraine, with the number IMB F-100138.

Morphological identification of the isolate was confirmed by the molecular analysis. The sequence of isolate 502 was determined and submitted to GenBank with accession number MK736305.1. The BLASTn analysis showed 99% similarity with 14 strains of *A. cucurbitacearum* and *P. melonis*.

Pathogenicity of *P. melonis* 502 to cucumber plants was confirmed. Symptoms of root system damage were observed after 14 days for cucumber plants grown in soil inoculated with this isolate (Figure 3). The cucumber variety Nizhynskyi 12 was very sensitive to the pathogen at the two true leaf growth stage (after 2 weeks from sowing) (Figure 3c). Above-ground symptoms were absent after 14 d, although severe symptoms were apparent on the root system at this time. Investigating the sensitivity of cucurbit species to Spanish *P. melonis* isolates, Armengol *et al.* (1998) found that cucumber plants ranged from resistant to very susceptible, and that, in field conditions, cucumber plants were less affected by *P. melonis* than melon plants with the same level of susceptibility. In contrast, Bruton *et al.* (2000b), in studies of

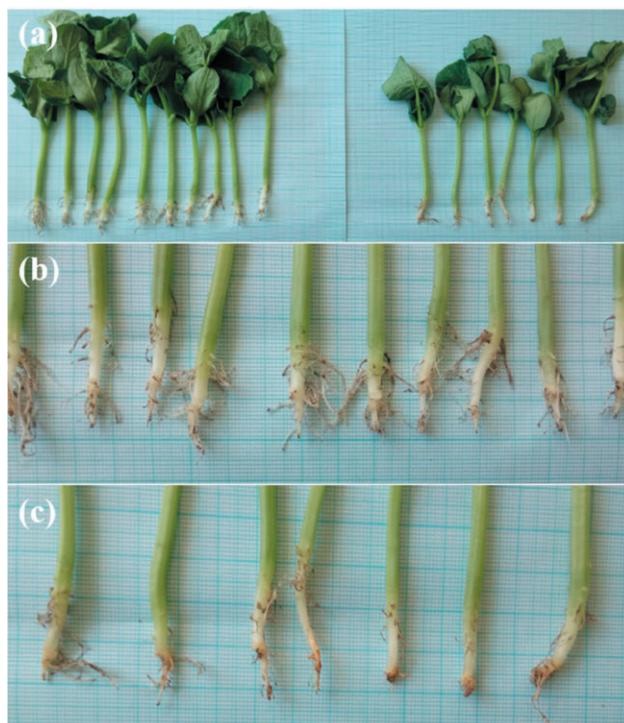


Figure 3. Healthy cucumber variety Nizhynskyi 12 plants (a - left, and b), and diseased plants (a - right, and c), after 14 d of cultivation.

pathogenicity of American *P. melonis* isolates, concluded that cucumber plants belonged to a high-resistance host group.

The present results are the first to show pathogenicity of *P. melonis* on cucumber plants in Ukraine. This study has confirmed the pathogenicity of this fungus to cucumber, and has shown that young cucumber seedlings were very susceptible to this pathogen.

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