

IDENTIFICATION OF *PLECTOSPHAERELLA MELONIS* ISOLATED FROM CUCUMBER PLANTS IN UKRAINE

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In the last ten years, many countries around the world recorded the cases of a new disease of the Cucurbits family group plants, the agent of which was identified as *Plectosphaerella melonis* (syn. *Acremonium cucurbitacearum*) (Garsia-Jimenez *et al.*, 1994; Bruton *et al.*, 1995; Gubler *et al.*, 1996; Alfaro-Garsia *et al.*, 1996; Cluck *et al.*, 1997; Armengol, 1997; Armengol *et al.*, 1998; Bruton, 1998; Bruton *et al.*, 1999; Bruton *et al.*, 2000a,b; Aergeten *et al.*, 2000; Martinez-Culebras *et al.*, 2004; Chilos *et al.*, 2008). The spectrum of host plants of *P. melonis* is limited. The fungus is pathogenic only for plants of the Cucurbitaceae family.

In 2012 *P. melonis* 502 strain as a probable pathogen was isolated from the affected cucumber plants grown indoors. The aim of our work was to identify the isolate on the basis of morphological-cultural and molecular-genetic characteristics and to confirm the pathogenicity of the isolate based on Koch's postulates.

The results of the research of the ability of the *P. melonis* fungus to cause a disease of cucumber plants are presented further. *P. melonis* 502 isolate was isolated from the highly affected plants grown in glass block greenhouses. The morphological and cultural characteristics of the fungus *P. melonis* 502 were described that allowed to classify it as the *P. melonis* species.

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 appliance to described methods (Birnboim & Doly, 1979; Chowdhury & Akaike, 2005; Chi *et al.*, 2009; Sika *et al.*, 2015). 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 BLAST analyses (<http://www.ncbi.nlm.nih.gov/blast>).

Using PCR method, the amplicon was obtained, with the length of 317 bp and phylogenetic analysis was performed. BLASTn searches at GenBank showed that the sequence of *P. melonis* 502 had 99% similarity with 14 isolates of *A. cucurbitacearum* and *Plectosphaerella melonis*. In addition, 99% of similarity with a typical strain of *A. cucurbitacearum* A-419 was established, that made it possible to attribute the strain 502 to *P. melonis* species. The Koch triad was reproduced in the work, namely the pathogenicity of *P. melonis* 502 on cucumber plants was confirmed, the pathogen was reisolated into a pure culture and the symptoms of the disease were described. The most sensitive to *P. melonis* 502 were young seedlings in 14 days after sowing the seeds in the soil, with the death of the lateral roots observed and the brown main root. After 28 days of cultivation, lesions of the root cervix were observed.

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.