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Histological Change in Cucumber Tissue and Cellulase Activity of *Plectosphaerella melonis* Strain 502

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Abstract: In the last ten years, many countries around the world recorded a new disease of the Cucurbitaceae, the agent of which was *P. melonis.* The ability of *P. melonis* 502 to form intracellular mycelium in the epidermal and parenchymal tissues of roots was shown. Leading tissues (xylem and phloem) did not colonize, which indicates the impossibility of plant vessel clogging and shows the fungus's biochemical effects on plants, which causes the process of pathogenesis. *P. melonis* 502 is able to develop in a wide range of pH values, while the pH-optimum is 8.5. *P. melonis* 502 is able to adjust the pH of the medium to the optimal value—8.5. We also showed that cellulase enzyme synthesis depends on pH. We studied the exo-, endo- and β-glucasidase activity of *P. melonis* 502 and found that the highest activity of cellulase enzymes was on a medium whose pH was 8.5. In the process, the total cellulolytic activity was 0.326 U mL^{-1}, exoglucanase activity—0.539 U mL^{-1}, endoglucanase activity—0.950 U mL^{-1} and β-glucosidase activity—0.795 U mL^{-1}.

Keywords: cellulase activity; cucumber; pathogen; pH-optimum; *Plectosphaerella melonis*; secondary metabolites

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1. Introduction

Cucumber (*Cucumis sativus* L.) is one of the most common vegetable crops. The yield of this crop is influenced by phytopathogenic micro-organisms: fungi, bacteria and viruses [1]. In recent times, many countries around the world recorded cases of a new disease of the Cucurbitaceae, the agent of which was *P. melonis* (syn. *Acremonium cucurbitacearum*) [1–19]. In Ukraine, this disease was on cucumber plants in 2012 [14,19]. *P. melonis* is a phytopathogenic fungus that is able to penetrate plant root tissues [3].

Pathogens' virulence factors are necessary for the micro-organism to colonize the tissues of the host plant and overcome the mechanisms of plant protection [20,21]. Parasitic fungi is due to the active development of fungal mycelium within the tissues of the host plant [22], which in turn is accompanied by the synthesis of secondary metabolites of the fungus, which will act as determinants of the disease [23].

To penetrate cells, pathogens can synthesize chemical compounds of different biological actions, including cell-wall-degrading enzymes (CWDEs). For example, cellulase enzymes take part in the degradation of the plant cellular wall [24–27]. For pathogenic micro-organisms, cellulases will initiate the process of pathogenesis and play the roles of pathogenic factors, helping them to penetrate the host plant [20,21]. In particular, it

has been shown that the synthesis of cellulase in vitro correlates with the degree of the disease [28,29] and highly aggressive isolates synthesize more cellulase in vitro than weakly aggressive isolates [30]. The culture fluid of phytopathogens which characterize cellulase activity in vitro can destabilize the cell membranes of plants [31].

Considering the above, the study of the role of metabolites of phytopathogens in plant–microbial interactions is a necessary condition for elucidating the mechanism of the pathological process. All previous investigations of *P. melonis* strains were aimed mainly at identifying and studying the spectrum of host plants and describing the symptoms of the disease. Recently, we reported that *P. melonis* 502 can synthesize phytohormone ethylene and regulate plant growth [19]. Here, we aimed to investigate *P. melonis* 502's ability to penetrate the root tissues of cucumber plants and synthesize cellulase enzymes that can act as virulence factors for the fungus in vitro.

2. Materials and Methods

2.1. Strain

Strain of *P. melonis* 502 was isolated from the affected root system of *C. sativus* (Koroliok variety) at the mass fruiting stage, which grew in glass block greenhouses (Chernihiv region, Ukraine). Strain deposited at the Depository of the Institute of Microbiology and Virology, NAS of Ukraine with the number IMB F-100138.

2.2. Artificial Infectious Background (AIB)

P. melonis 502 was cultivated in 1 L flasks with a capacity on a solid substrate, which included: oat seeds, oatmeal, water, chalk and gypsum. Sterilization of the substrate was performed twice at a temperature of 128 °C and a pressure of 1.5 ATM for 1 h 30 min. The inoculum was a pure culture of the *P. melonis* 502, which was isolated on a solid nutrient medium wort agar from affected cucumber plants and was obtained by washing conidia and fragments of fungal hyphae from wort agar. The flasks were kept in a thermostat at a temperature of 26 ± 2 °C. After overgrowing the substrate with mycelium (21st day), it was transferred to paper bags, dried to a constant mass at 30 °C and crushed. To determine the titer of infectious material, we used Goryaev's chamber and carried out sowing on wort agar (4–5% dry matter).

2.3. Plant Material

The study of the ability of *P. melonis* 502 to colonize the root system of *C. sativus* (Koroliok variety) was tested in a growing experiment. Plastic containers (2 L) were filled with 1800 g of sterile soil. Soil: sod-medium podzolic dusty-sandy; humus content—1.02%; nitrogen—54.9 mg/kg; mobile forms of phosphorus—110–120 mg of P_2O_5, potassium exchange—120–130 mg of K_2O per 1 kg of soil; pH—6.0; Ca—5.8, Mg—0.61 mg per 100 g of soil. At a depth of 2–3 cm, 10 g of infectious material was made (50 thousand CFU per 1 g of soil), and seeds were sown in each pot. After emergence, the pots were thinned to 5 plants per 1 pot. The plants were grown in a growing house for 6 weeks with natural lighting. Soil moisture was maintained at 60% of total moisture content. The experiment was repeated three times.

2.4. Histological Processing of Samples and Stain Preparation

For microscopic examination, the roots were pre-washed, stained and fixed. With a thin blade, we cut the roots of plants directly by hand into small segments 5–7 mm long. The culture of fungus was detected by staining the affected root tissues by the Kobel method: longitudinal and cross sections of living plant roots were placed in a lactic acid solution of aniline blue (0.1 g of aniline blue, 50 mL of lactic acid and 100 mL of water) for 5 min, and then the sample was placed in 40% lactic acid for differentiation followed by washing the sample in distilled water. Hyphae of fungi were painted blue [32]. Histological changes in the tissues of the root system of cucumber plants were observed on temporary

micropreparations using light microscope MC 200CT ("Micros", Gewerbezone, Austria) and camera Sony DSC-S650.

2.5. pH Growth

The *P. melonis* 502 was cultured on BMA (barley meal agar) with different pH indexes: 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.0, 11.5 and 12.0 at 26 °C. On the 10th day, the diameter of the colonies in two mutually perpendicular directions and the radial growth rate was determined [33].

2.6. Physiological Phases of Growth

The *P. melonis* 502 was cultivated (220 rpm (rotations per minute)) for 21 days at 26 °C on modified Czapek medium: 20 g glucose, 2 g $NaNO_3$, 1 g K_2HPO_4, 0.5 g $MgSO_4 \times 7H_2O$, 0.5 g KCl and 0.01 $FeSO_4 \times 7H_2O$ per liter, pH—6.0 [34]. Nutrient medium was inoculated by spores suspension (T = 1×10^6 CFU) in the amount of 5% of volume. The amount of biomass was determined gravimetrically (by drying the fungus to constant weight for 5 h at 105 °C [33]. The change in the pH values of the cultural fluid were recorded with a universal pH meter pH-150 MA.

2.7. Cellulase Assay

The *P. melonis* 502 were cultivated in test tubes (in a static culture) containing 5 mL of modified Czapek medium: 2.5 g $(NH_4)_2HPO_4$, 1.0 g K_2HPO_4, 0.5 g $MgSO_4$ and 0.5 g KCl, 0.01 $FeSO_4$ with different pH values—5.5, 7.0 and 8.5. The only source of carbon was a strip of filter paper (Munktell & Filtrak GmbH, Bärenstein, Germany), weighing 50 g (1×6 cm), that was placed in each test tube. Nutrient medium was inoculated by spores suspension (T = 1×10^6 CFU) in the amount of 5% of volume and was cultivated in the thermostat in dark conditions at 26 °C for 8 weeks. The culture fluid (supernatant) was obtained by filtration.

Total cellulose and exoglucanase activities were assayed by mixing 1 mL supernatant with 50 mg filter paper and avicel (Evalar, Biysk, Russia), respectively, in 1 mL 0.05 M citrate–phosphate buffer with different pH values (5.5, 7.0 and 8.5) and incubating it in a water bath at 40 °C for 60 min. Endoglucanase and β-glucosidase activities were evaluated by mixing 1 mL supernatant with 1 mL 0.5% CMC and 0.025% cellobiose (Merck KGaA, Darmstadt, Germany) in 0.05 M citrate–phosphate buffer with different pH values (5.5, 7.0 and 8.5), respectively, and incubating it in a water bath at 40 °C for 30 min.

Release of reducing sugars was assessed by the Somogyi method [35] modified from Nelson procedure [36] with glucose as standard. A total of 1 mL of Somogyi reagent was added to 2 mL of reaction mixture and incubated at 100 °C for 15 min. Then, the mixture cooled on an ice water bath and 1 mL of Nelson reagent was added. The volume was filled up to 25 mL with water and mixed. Reducing sugars were determined on photoelectric colorimeter at 560 nm.

One unit (U) of enzyme activity was defined as the number of enzymes required to liberate 1 μmol of reducing sugar for min in mL^{-1} expressed as glucose equivalents. Cellulase activity was measured every 7 days. Repetition of experiment is triplicate.

2.8. Statistical Methods

The significance of the experimental data was analyzed using STATISTICA 12 software (StatSoft, Hamburg, Germany). The distribution of data checked for normality using the Shapiro–Wilk W-test and the homogeneity of variance checked using the Brown–Forsythe test. The significance of the differences was measured using One-way Analysis of Variance (ANOVA). Particularly, Duncan Multiple Range Test (DMRT) was used to check if each parameter differed significantly under different values of studied factors. The level of significance was set to $p < 0.05$.

3. Results and Discussion

3.1. Histopathology of Infections Caused by P. melonis 502 on the Root Tissues of Cucumber

After 6 weeks of growing the plants on infected soil, the cortex cells had a distorted appearance (Figure 1a). *P. melonis* 502 densely colonized the parenchymal and epidermal tissues of the root. Hyphae were of different thicknesses and were observed not only in the intercellular space but also inside the cells, forming intracellular mycelium (Figure 1b–d). Figure 1f shows the hyphae of the fungus inside the cell, as well as the necrotic cell walls of root cells, which indicates the synthesis of hydrolytic enzymes by the fungus, providing the degradation of cells (Figure 1f).

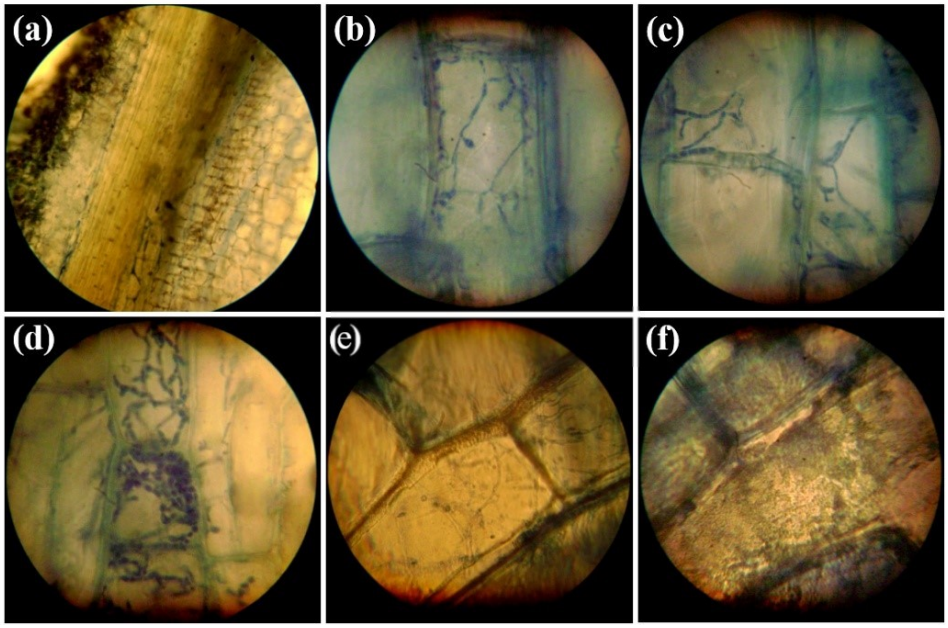

Figure 1. Longitudinal section of the main roots of cucumber plants affected by *P. melonis* 502 (**a**). Cross section of the main cucumber roots infected with *P. melonis* 502 (**b**–**f**). Colonization by the fungus *P. melonis* 502 of the roots of cucumber plants, which leads to necrosis of the cell wall (**e**). Lysis of root cells of cucumber plants affected by *P. melonis* 502 (**f**). 900× magnification.

The leading tissues (xylem and phloem) did not colonize (Figure 1a), which indicates the impossibility of plant vessel clogging and shows the fungus's biochemical effects on plants, which causes the process of pathogenesis, but in xylem there were tiloses (Figure 2a). Tiloses were also present in the roots of plants affected by the fungi *Phaemoniella chlamydospora* and *Phaeoacremonium* sp. [37]. Our results confirm the results of Alfaro-Fernández and García-Luis [3], who also recorded the presence of tiloses in leading root tissues in the defeat of *A. cucurbitacearum* (syn. *P. melonis*). The etiology of tiloses formation is quite ambiguous and debatable, but it is known that these structures reduce water supply to the plant under the conditions of high demand for moisture, which may be the reason for their depletion [3,38]. In addition, we found microsclerotia in the affected tissues of the roots of cucumber plants (Figure 2b), which completely fill the cell of the host plant. Microsclerotia are known to increase the survival of a micro-organism in soil under adverse environmental conditions [39].

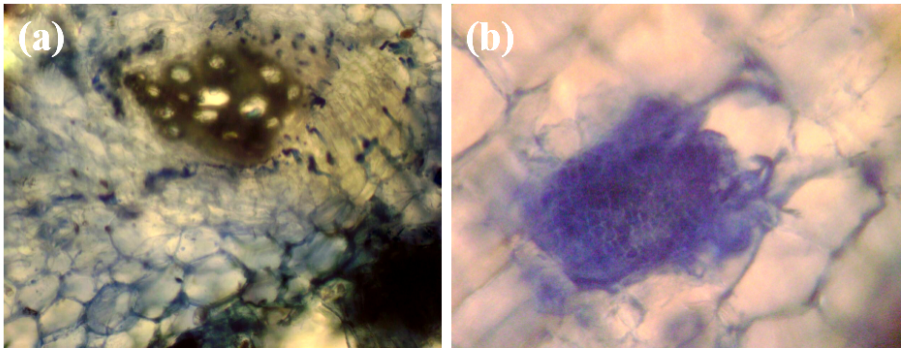

Figure 2. Cross section of cucumber root tissue affected by *P. melonis* 502. (**a**) Tiloses formed on the inner surface of xylem vessels, 200× magnification. (**b**) Microsclerotia of *P. melonis* in root tissues of cucumber plants, 400× magnification.

We previously reported that *P. melonis* 502 can synthesize the phytohormone ethylene (ET) in vitro and regulate plant growth [19]. Phytohormones can promote the penetration of the pathogen into the tissues of host plants, create a niche for the pathogen and/or trigger other disease processes [40]. ET activates and accelerates the aging process of plant tissues [41]. At the same time, that tissue shows increased activity to cellulase, which leads to the destruction of intercellular connections [42]. ET modulates plant susceptibility and fungal growth in plant tissue also [43].

3.2. pH Growth of P. melonis 502

P. melonis 502 on BMA grows in the pH ranges from 6.0 to 12.0 (Figure 3, Table 1) and refers to acid-tolerant micro-organisms, that is, those that are able to grow and develop within wide pH ranges. The pH-optimum is slightly alkaline (pH 8.5), with the colony diameter on the 10th day being 24.8 ± 0.1 mm (Table 1) and with the radial growth rate being 0.103 mm per h. The ability of the Plectosphaerellaceae fungi to grow in alkaline conditions was shown earlier [44]. Bondarenko et al. [45] isolated the *Plectosphaerella* spp. from acidic sod-podzolic and neutral soils by sowing on alkaline agar (pH 10.0–10.5). The ability of these fungi to grow on nutrient media with pH 4.0–10.0 is shown. Our results confirm that *P. melonis* 502 is alkalophilic.

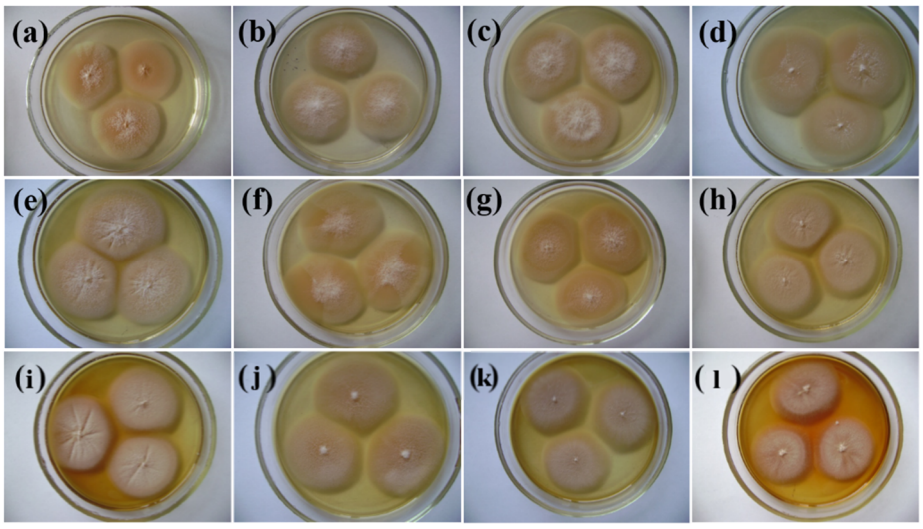

Figure 3. *P. melonis* 502 colonies on BMA with different pH values: 6.0 (**a**), 6.5 (**b**), 7.0 (**c**), 7.5 (**d**), 8.0 (**e**), 8.5 (**f**), 9.0 (**g**), 9.5 (**h**), 10.0 (**i**), 10.5 (**j**), 11.0 (**k**) and 11.5 (**l**).

Table 1. Growth rate of *P. melonis* 502 colonies at different pH values.

pH	Colony Diameter, mm (10 days)	Radial Growth Rate, mm per h
6.0	20.3 ± 0.2 e	0.084 ± 0.000 e
6.5	21.0 ± 0.0 e	0.088 ± 0.000 e
7.0	23.1 ± 0.1 c	0.096 ± 0.000 c
7.5	23.6 ± 0.2 b	0.098 ± 0.001 b
8.0	24.0 ± 0.0 b	0.100 ± 0.000 b
8.5	24.8 ± 0.1 a	0.103 ± 0.001 a
9.0	23.9 ± 0.1 b	0.100 ± 0.000 b
9.5	23.8 ± 0.2 b	0.099 ± 0.001 b
10.0	23.1 ± 0.1 c	0.096 ± 0.001 c
10.5	23.2 ± 0.1 bc	0.097 ± 0.001 bc
11.0	21.9 ± 0.1 d	0.091 ± 0.000 d
11.5	20.7 ± 0.2 e	0.086 ± 0.001 e
12.0	14.7 ± 0.3 f	0.061 ± 0.001 f

The values represent the average ± SD of three replicates. Mean growth rates in the same column followed by different letters are significantly different according to Duncan's multiple range test at $p \leq 0.05$.

3.3. Physiological Growth Phases of *P. melonis* 502

The stationary phase of *P. melonis* 502 occurred after 10 days (Figure 4b). We also observed that the alkalescencing of the nutrient medium occurred during the stationary phase (up to 14 days) until a pH of 8.5 was reached (Figure 4a). Upon further cultivation of *P. melonis* 502, the pH of the medium in the deep culture remained unchanged.

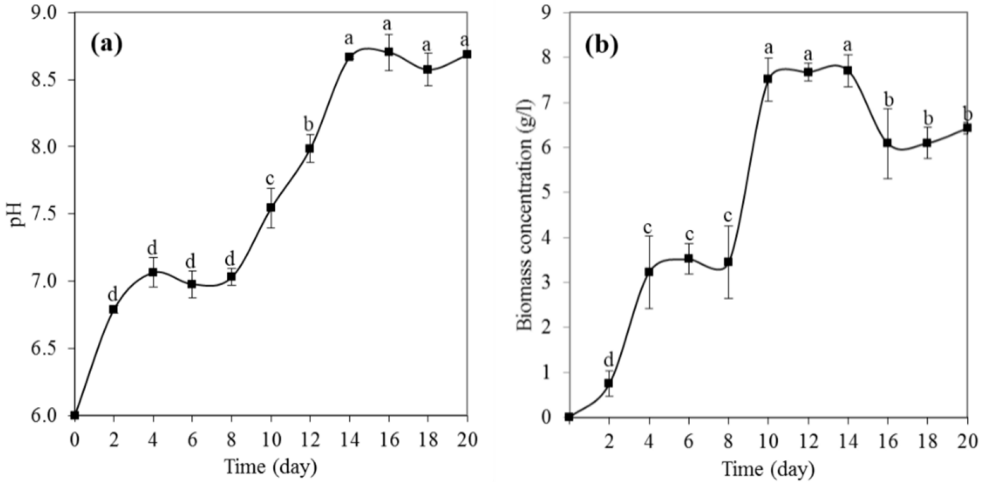

Figure 4. pH of the *P. melonis* 502 cultural fluid (**a**) and biomass concentration (**b**) in shake cultures. The values represent the average ± SD of three replicates. Mean growth rates followed by different letters are significantly different according to Duncan's multiple range test at $p \leq 0.05$.

Pathogens can adapt their broad pathogenic factors arsenal to a particular host during the evolution process and are able to affect and regulate the pH environment, since this indicator influences the activity of many enzymes, including enzymes of degradation of the cellular walls of plants [46,47]. We have shown that *P. melonis* 502 alkalizes the nutrient medium to the optimum pH for growth (pH 8.5). Jennings [48] believes that environmental leaching happens due to the active ammonia formation by amino acid deamination. Some fungi, such as *Polyporus tricholoma,* increased the pH of the nutrient medium with a different pH level to 5.5, regardless of the initial pH and other factors [49]. This ability to increase the pH of the nutrient medium was shown for some pathogenic fungi, in particular *Aspergillus nidulans* [47], *Candida albicans* [50] and *Metarhizium anisopliae* [51]. The phytopathogen of persimmon fruit *A. alternata* increased the pH of the nutrient medium from 3.8 to 6.0, which in turn induced the expression of the AaK1 gene and synthesis of endo-1,4-β-glucanase, which plays an important role in the development of the disease [52]. Adjusting the pH of

the environment by *Colletotrichum gloeosporioid* in the host plant tissues would depend on the buffering power of the cytoplasm, and therefore, each specific pathogen would have different effects on different host plants [53].

3.4. Cellulase Assay

Maximum total (Table 2), exoglucanase (Table 3), endoglucanase (Table 4) and β-glucosidase (Table 5) activities developed after 6 weeks of cultivation. At the same time, pH 8.5 of the medium was the most optimal for the synthesis of cellulase enzymes. The maximum total cellulase activity was 0.326 U/mL^{-1}, exoglucanase—0.539 U/mL^{-1}, endoglucanase—0.950 U/mL^{-1} and β-glucosidase was 0.795 U/mL^{-1}.

Table 2. Total cellulase activity of *P. melonis* 502 under different pH values.

Cultivation Period, Week	Activity (U mL^{-1}) ± SD pH		
	5.5	**7.0**	**8.5**
1	0.031 ± 0.002 aa	0.047 ± 0.002 aa	0.077 ± 0.005 ab
2	0.020 ± 0.001 aa	0.025 ± 0.000 aa	0.053 ± 0.005 ab
3	0.046 ± 0.004 aa	0.065 ± 0.006 ba	0.118 ± 0.004 bb
4	0.048 ± 0.002 aa	0.071 ± 0.002 ba	0.132 ± 0.006 bb
5	0.066 ± 0.005 aa	0.072 ± 0.003 ba	0.163 ± 0.006 cb
6	0.050 ± 0.005 aa	0.068 ± 0.010 ba	0.326 ± 0.019 db
7	0.066 ± 0.023 aa	0.039 ± 0.013 ca	0.145 ± 0.011 eb
8	0.021 ± 0.001 ba	0.048 ± 0.006 cb	0.149 ± 0.007 ec

The values represent the average ± SD of three replicates. Mean growth rates in the same column followed by different letters are significantly different according to DMRT test at $p \leq 0.05$. The first letter shows the differences between the weeks of cultivation, and the second letter shows the differences between the pH values of the medium.

Table 3. Exoglucanase activity of *P. melonis* 502 under different pH values.

Cultivation Period, Week	Activity (U mL^{-1}) ± SD pH		
	5.5	**7.0**	**8.5**
1	0.121 ± 0.003 aa	0.128 ± 0.002 aa	0.135 ± 0.010 aa
2	0.080 ± 0.000 ba	0.090 ± 0.002 ba	0.141 ± 0.017 ab
3	0.101 ± 0.013 ba	0.161 ± 0.003 cb	0.253 ± 0.002 bc
4	0.068 ± 0.003 ca	0.084 ± 0.001 da	0.151 ± 0.001 cb
5	0.115 ± 0.005 da	0.142 ± 0.003 ea	0.248 ± 0.010 db
6	0.148 ± 0.015 ea	0.225 ± 0.005 fa	0.539 ± 0.019 ec
7	0.136 ± 0.006 ea	0.097 ± 0.012 gb	0.156 ± 0.003 fd
8	0.063 ± 0.004 fa	0.095 ± 0.004 gb	0.196 ± 0.010 gc

The values represent the average ± SD of three replicates. Mean growth rates in the same column followed by different letters are significantly different according to DMRT test at $p \leq 0.05$. The first letter shows the differences between the weeks of cultivation, and the second letter shows the differences between the pH values of the medium.

Table 4. Endoglucanase activity of *P. melonis* 502 under different pH values.

Cultivation Period, Week	Activity (U mL^{-1}) ± SD pH		
	5.5	**7.0**	**8.5**
1	0.061 ± 0.001 aa	0.059 ± 0.001 aa	0.069 ± 0.002 aa
2	0.037 ± 0.001 aa	0.041 ± 0.001 aa	0.067 ± 0.003 aa
3	0.066 ± 0.000 aa	0.139 ± 0.019 bb	0.272 ± 0.016 bc
4	0.084 ± 0.000 aa	0.127 ± 0.022 ba	0.337 ± 0.011 cb
5	0.024 ± 0.001 aa	0.090 ± 0.008 ba	0.404 ± 0.005 db
6	0.000 ± 0.000 aa	0.016 ± 0.022 bb	0.950 ± 0.071 eb
7	0.000 ± 0.000 aa	0.025 ± 0.009 ba	0.233 ± 0.004 fb
8	0.039 ± 0.002 aa	0.049 ± 0.003 ba	0.421 ± 0.043 gb

The values represent the average ± SD of three replicates. Mean growth rates in the same column followed by different letters are significantly different according to DMRT test at $p \leq 0.05$. The first letter shows the differences between the weeks of cultivation, and the second letter shows the differences between the pH values of the medium.

Table 5. β-glucosidase activity of *P. melonis* 502 under different pH values.

Cultivation Period, Week	Activity (U mL^{-1}) ± SD pH		
	5.5	**7.0**	**8.0**
1	0.059 ± 0.001 aa	0.078 ± 0.014 aa	0.212 ± 0.006 ab
2	0.036 ± 0.002 aa	0.043 ± 0.005 aa	0.174 ± 0.005 ab
3	0.066 ± 0.000 aa	0.161 ± 0.006 bb	0.244 ± 0.016 bc
4	0.090 ± 0.002 aa	0.195 ± 0.010 bb	0.293 ± 0.004 bc
5	0.168 ± 0.007 ba	0.245 ± 0.009 bb	0.421 ± 0.024 cc
6	0.363 ± 0.042 ca	0.283 ± 0.006 bb	0.795 ± 0.034 dc
7	0.203 ± 0.019 da	0.127 ± 0.018 cb	0.398 ± 0.064 ec
8	0.058 ± 0.013 ea	0.106 ± 0.003 ca	0.389 ± 0.012 eb

The values represent the average ± SD of three replicates. Mean growth rates in the same column followed by different letters are significantly different according to DMRT test at $p \leq 0.05$. The first letter shows the differences between the weeks of cultivation, and the second letter shows the differences between the pH values of the medium.

Phytopathogenic and entomopathogenic fungi of the genus *Colletotrichum* showed a dependence on the pH of the nutrient medium, in particular, the synthesis of cellulases at pH 8.0 was higher than at 5.0 and 6.8 [54]. For phytopathogen *F. oxysporum*, the optimal pH was 6.0 [55]. Hemibiotrophic pathogen of the anthracnose of beans *C. lindemuthianum* synthesized a complex of cellulase enzymes at an optimal pH of 5.5 [56]. Furthermore, a correlation between cellulase activity and the virulence of *Sclerotinia sclerotiorum* isolates was shown [24]. The biosynthesis of cellulase and pectinase plays an important role in the pathogenesis of *Alternaria brassicae* [26] and *A. alternata* [25]. Tomato phytopathogens *A. tenuissima*, *A. arborescens*, *A. mimicula*, *A. interrupta* and *A. infctoria* synthetized pectinase, cellulase, amylase, protease and lipase; however, only cellulase and pectinase production have correlated with isolate pathogenicity [57]. The role of cellulase enzymes in the pathogenesis of the *Cephalosporium acremonium*, which causes root rot of corn plants, proved to be important. For example, the pathogenicity of the fungus and crop loss correlated with the synthesis of hydrolytic enzymes, including cellulose [58].

Some fungi have exoglucosidase activity. For example, endophytic fungus *Chaetomium cochliodes* 3250 was characterized by exoglucanase activity along with endoglucanase and β-glucosidase [59]. However, the specified activities are much lower than those of *P. Melonis* 502, even with a pH of 5.5 and 7.0, which are not optimal for *P. melonis* 502's growth and development. It should be noted that the exoglucosidase activity curve of *P. melonis* 502 is wave-like. We have also registered an increase in exocellulase activity between weeks 3 and 6 of cultivation. This, in turn, proves that fungi use reducing sugars as a carbon source [60]. When Ramanathan et al. [55] studied the cellulase activity of phytopathogen *F.*

oxysporum, they also showed two peaks of cellulase activity—at days 4 and 8 of cultivation on a rotary shaker. In addition, in vitro synthesis of enzymes varies depending on the method of cultivation. High cellulase and β-glucosidase activity of the phytopathogen of sugar beet *Sclerotium rolfsii* was shown, the peaks of which occurred on days 9 and 10, respectively, while in a dynamic culture, the activity of enzymes was almost five times higher than in a static culture [27,61,62]. Regarding *Plectosphaerella*, it is known that the pH-optimum of *P. cucumerina* for the xylanase is 6.0 [63]. Chitinases *Plectosphaerella* sp. strain MF-1 is most active at pH 3–4 [64].

In general, it is now known that the *P. melonis* 502 is capable of the synthesis of ethylene [19] and cellulase enzymes in vitro. We also found that the culture fluid can regulate plant growth [19] and the fungus itself can penetrate into the tissues of plant roots. However, additional research is needed to reveal the mechanism of pathogenesis and to establish pathogenicity and virulence factors.

4. Conclusions

Thus, we have shown that *P. melonis* 502 is able to penetrate the root tissues of plants and form intracellular mycelium. It affects mainly cortex tissues (epidermal and parenchymal). Leading tissues (xylem and phloem) are not colonized, which indicates the impossibility of plant vessel blockage and indicates the effect of fungal metabolites on plants, which determine the process of pathogenesis. *P. melonis* 502 is able to develop in a wide range of pH, while the pH-optimum is 8.5. *P. melonis* 502 is able to adjust the pH of the medium to the optimal value—8.5. We also showed that the synthesis of cellulase enzymes depends on the pH. We studied the exo-, endo- and β-glucosidase activities of *P. melonis* 502 and found that the highest enzyme activity was shown on pH 8.5. We noted cells with intracellular mycelium and destroyed cell walls. Cellulase enzymes may be virulence factors and can degrade the plant cell wall.

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