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FILAMENTOUS FUNGI AS PRODUCERS OF EXTRACELLULAR HYDROLYTIC ENZYMES

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Abstract. Filamentous fungi contribute in the degradation of organic material and help for attaching various surfaces through secretion of extracellular hydrolytic enzymes. However, the enzymatic capacity of numerous species of Dothideomycetes associated with plants stay insufficiently characterrized. In this study, five filamentous species of fungi, isolated in pure culture from infected plant leaves (*Atriplex patula, Cannabis sativa, Chelidonium majus* and *Sambucus nigra*). Were identified as *Pseudocercospora depazeoides* (strain 1), *Septoria cannabis* (2), *Septoria chelidonii* (3), *Cercospora chenopodii* (4) and *Alternaria chartarum* (5). The study of extracellular proteolytic and glycosidic activities were carried out in culture liquid supernatants. None of the istrains tested hydrolyzed elastin, fibrin, fibrinogen or collagen, while keratinolytic activity was detected in one mycelial culture. β-D-Glucosidase activity was observed in all tested fungi, whereas α-L-rhamnosidase activity was detected only in one strain, The results indicate that the associated with plants fungi could may be of interest for the study of other enzymatic activities
Keywords: *Septoria, Cercospora*, *Pseudocercospor,* glycosidases, keratinase.

Filamentous fungi are important components of plant-associated organism communities. By colonizing plant tissues, they participate in the transformation of plant-derived organic matter and contribute to nutrient cycling. The ability to exploit different ecological niches is closely associated with extracellular enzymes that release low-molecular-weight compounds from complex biopolymers. The fungal enzymes, in particualitly extracellular proteases and glycosidases have both ecological and physiological significance, and also are intensively investigated as potential biocatalysts for food, pharmaceutical, agricultural and biomass-processing technologies (Scherlach & Hertweck, 2021; Lübeck & Lübeck, 2022). Their production is particularly important for fungi as the plant tissues contain structurally complex polymers and diverse secondary metabolites. Proteolytic enzymes participate in protein turnover and nutrient acquisition, whereas glycosidases contribute to the utilization of plant carbohydrates and glycosylated compounds. So, β-D-glucosidases are involved in the final stages of cellulose hydrolysis and are of interest for lignocellulosic biomass conversion, while α-L-rhamnosidases can remove terminal rhamnose residues from glycosides and are relevant to the biotransformation of plant flavonoids and aroma precursors (Lübeck & Lübeck, 2022). Keratinases are the other specialized group of extracellular proteases capable to degrade the highly resistant keratin structures and are perspective in biological processing of keratin-containing wastes (Alwakeel et al., 2021). The aim of this study was to characterize the extracellular proteolytic and glycosidase potential of filamentous fungi and to identify isolates showing enzymatic activity.

Materials and Methods. Studied fungi were isolated from damaged leaves of *Atriplex patula, Cannabis sativa, Chelidonium majus* and *Sambucus nigra,* collected in the Kyiv region in 2024. Pure cultures were maintained on potato dextrose agar at 25 °C. Proteolytic

activity was assessed in culture liquid supernatants using elastin, fibrin, fibrinogen, collagen, casein and keratin as substrates. α-L-Rhamnosidase and β-D-glucosidase activities were determined using p-nitrophenyl-α-L-rhamnopyranoside and *p*-nitrophenyl-β-D-glucopyranoside, respectively. Naringin and neohesperidin was also used to determine α-L-rhamnosidase activity. The identification of fungi was based on macro- and micromorphological characteristics, including light microscopy and scanning electron microscopy. Enzyme assays were performed in 5–7 replicates, and differences were evaluated statistically at $P < 0.05$.

Results and Discussion. Fungal isolates isolated from the leaves of various plants (*Atriplex patula, Cannabis sativa, Chelidonium majus* and *Sambucus nigra,*) were identified as *Pseudocercospora depazeoides* (strain 1), *Septoria cannabis* (2), *Septoria chelidonii* (3), *Cercospora chenopodii* (4) and *Alternaria chartarum* (5). Thus, the enzymatic screening covered representatives of several genera of Dothideomycete and observed enzymatic activities expand the available information on the physiological characteristics of these plant-associated fungi.

All studied strains significantly differed in their extracellular enzymatic profiles. None of the tested cultures demonstrated detectable hydrolysis of elastin, fibrin, fibrinogen or collagen, remained whereas at trace levels exerted caseinolytic activity. Keratinolytic activity was detected only in *Alternaria chartarum* (5), reaching 4 U/ml. This result is importand because keratin is a structurally resistant protein stabilized by disulfide bonds, hydrogen bonding and hydrophobic interactions, and fungal keratinases are therefore of interest for environmentally compatible processing of keratinous residues.

In contrast to the limited proteolytic activity, glycosidase activity was detected more broadly. All tested strains produced β-D-glucosidase, with activities ranging from 0.2 to 0.4 U/ml. The highest values, 0.4 U/ml, were observed for from *Pseudocercospora depazeoides* (1) and *Septoria cannabis* (2). Only strains from *Pseudocercospora depazeoides* produced α-L-rhamnosidase, with an activity of 0.37 U/ml on the synthetic substrate. This strain 1 also hydrolyzed the natural substrates naringin and neohesperidin, with activities of 0.35 and 0.20 U/ml, respectively. The lower activity toward natural substrates compared with the synthetic substrate suggests differences in substrate accessibility or enzyme–substrate interaction. Nevertheless, hydrolysis of naringin and neohesperidin demonstrates the ability of this fungal strains to act on naturally occurring rhamnosylated flavonoids.

Conclusions. The studied filamentous fungi showed low or undetectable activity toward the tested protein substrates but displayed a more pronounced activity to glycosides. Particularly noteworthy are the keratinase activity of *A. chartarum* and the α-L-rhamnosidase activity of *P. depazeoides*. β-D-Glucosidase activity was detected in all isolates, with the highest activity in *P. depazeoides* and *S. cannabis*, that indicates a broader capacity for transformation of plant-derived glycosides by studied fungi. These findings indicate that less-studied plant-associated fungi may represent useful sources of extracellular hydrolases and justify further investigation of their enzyme systems, including optimization of cultivation conditions and biochemical characterization of the most active enzymes.

References

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