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PPECTINOLYTIC ACTIVITY OF THE MACROMYCETE *Trametes hirsuta* (Wulfen) Lloyd UNDER SURFACE AND SUBMERGED CULTIVATION

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Pectinolytic enzymes are of considerable industrial importance due to their ability to degrade pectin in plant cell walls. Recently, increasing attention has been directed toward basidiomycetes, particularly the genus Trametes, which are capable of synthesizing complex enzymatic systems; however, their potential for pectinase production remains insufficiently explored.

Aim. The study purposed to investigate the growth and enzymatic activity of the basidiomycete *Trametes* hirsuta under surface and submerged cultivation in order to expand the biotechnological potential of these organisms as pectinase producers.

Materials and Methods. The study involved six strains of the macromycete *Trametes hirsuta* deposited at the Institute of Botany of the National Academy of Sciences of Ukraine. A peptone–yeast extract medium supplemented with pectin (solid nutrient medium) was used for surface cultivation. Mycelial growth was measured daily, and pectinase activity was assessed using a cetrimide-based method. Submerged cultivation was performed in a glucose–peptone–yeast extract medium for 14 days, after which protein content, dry biomass, and pectinolytic enzyme activity were analyzed. Statistical analysis included Student’s t-test and Duncan’s multiple range test using R and Excel software.

Results. *T. hirsuta* strains 5018 and 5137 demonstrated more active growth on pectin-containing media. Overall, the overgrowth period ranged from 5 to 9 days, depending on the strain. The highest radial growth rate was recorded for strain *T. hirsuta* 1569 (10.25 mm/day). The pectinase activity index (PAI) was determined, with strain *T. hirsuta* 1569 exhibiting the highest value (1.08 ± 0.04), indicating its strong potential for pectinolytic enzyme synthesis.

Conclusions. During submerged cultivation, strain *T. hirsuta* 1569 showed high polygalacturonase activity (1.28 ± 0.13 U/mL) and pectinesterase activity (0.31 ± 0.04 U/mL). In contrast, strain *T. hirsuta* 338 exhibited significantly lower enzymatic activities and was therefore excluded from further experiments.

Keywords: *Trametes*, pectinolytic enzymes, growth rate, pectinase activity, polygalacturonase, pectinesterase, biotechnology.

Pectinolytic enzymes (pectinases) constitute one of the key groups of industrial enzymes responsible for the depolymerization of pectin, the principal component of plant cell walls [1]. They catalyze the breakdown of complex pectic polysaccharides into low-molecular-weight products, such as galacturonic acid. The main mechanisms of pectinase action include hydrolysis, β-elimination (trans-elimination), and de-esterification [2]. According to their specificity toward the pectin backbone, pectinolytic enzymes are classified into several groups. The major classes include pectinesterases (PE), which reduce the degree of pectin methylesterification, and polygalacturonases (PG), which hydrolyze glycosidic bonds between galacturonic acid residues [3].

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Due to their multifunctional catalytic properties, pectinases are widely applied in the food industry (juice, wine, and puree production) [4], as well as in the textile industry, agro-industrial waste processing, wastewater treatment, and paper manufacturing [5].

Most commercially available pectinases are produced by microorganisms such as *Aspergillus niger*, *Trichoderma viride*, and *Penicillium* spp. via submerged or solid-state fermentation [4, 6]. *Aspergillus* and *Penicillium* species possess GRAS status (Generally Recognized As Safe for food applications), which makes them the most commonly used organisms for industrial pectinase production [7]. Bacterial producers are also widely employed in industrial enzyme manufacturing [8]. *Serratia marcescens*, *Lysinibacillus macrolides*, and representatives of the genera *Bacillus*, *Pseudomonas*, *Erwinia*, and *Streptomyces* exhibit high levels of pectinolytic enzyme synthesis. The highest pectinolytic activity among bacteria is observed in members of the genera *Bacillus* and *Erwinia* [9–11].

At present, the search for alternative enzyme sources remains an important scientific challenge [12, 13]. Recently, increasing attention has been directed toward basidiomycetes, wood-decaying fungi capable of producing complex enzymatic systems, including ligninolytic enzymes involved in lignocellulose degradation [14–16].

Xylotrophic basidiomycetes, including *T. hirsuta*, colonize a wide range of plant substrates, particularly wood [17]. The plant cell wall represents the primary target of fungal activity. Its main structural components-cellulose, hemicellulose, lignin, and pectin-are therefore subject to enzymatic degradation during macromycete growth [18]. The efficiency of polysaccharide biodegradation depends on the activity of extracellular enzymes responsible for the hydrolysis of these polymers, including pectin [19].

The synthesis of pectinolytic enzymes is regulated by the expression of corresponding genes. Basidiomycetes possess approximately 11 carbohydrate-active enzyme (CAZyme) gene families involved in pectin degradation [20], among which glycoside hydrolases (GH) predominate [21]. In *T. hirsuta*, the presence of the genes GH3, GH105, GH2, GH35, GH43, GH51, PL4, CE1, and CE8 has been confirmed [22].

Among macromycetes of the genus *Trametes* (*T. trogii*, *T. versicolor*, *T. hirsuta*), the synthesis of polygalacturonase and pectin lyase has been partially investigated; however, the number of such studies remains limited [23–26]. Despite the wide application of pectinolytic enzymes, the potential of wood-decaying basidiomycetes for their production is still insufficiently explored. A deeper understanding of their enzymatic activity and optimal cultivation conditions is required.

The aim of this study was to investigate the growth and enzymatic activity of the basidiomycete *T. hirsuta* under surface and submerged cultivation.

Materials and Methods

Objects

The study objects were six macromycete strains of *Trametes hirsuta* deposited in the Mushroom Culture Collection of the M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine (IBK): 338, 358, 359, 1569, 5018, and 5137 [27].

Culture media

The *T. hirsuta* strains were cultivated on a peptone–yeast extract medium supplemented with 0.5% pectin (PYPA) [28, 29]. The initial pH was adjusted to 7.0 ± 0.2 using 1 N KOH and HCl solutions. A 7-day-old mycelial disc of the corresponding strain was placed in the center of a 90 mm Petri dish containing the medium, and incubation was carried out at 28 ± 1 °C until the plate surface was fully colonized. During cultivation, the mycelial radius was measured daily, and the radial growth rate (RGR) was calculated [30].

Methods for solid-state cultivation

Primary screening of pectinase-producing strains was performed on PYPA medium. At the end of cultivation, Petri dishes were flooded with a 1% solution of cetyltrimethylammonium bromide. Subsequently, the pectinase activity index was calculated using the following formula:

$$PAI = \frac{D_H}{D_C},$$

where DH and DC are the diameters of the halo and the colony, respectively (mm) [31].

Methods for cultivation in liquid stationary culture

Submerged cultivation was carried out under stationary conditions in a liquid glucose–peptone–yeast extract medium with an initial pH of 7.0 ± 0.2. Inoculation was performed by introducing three agar discs from a 7-day-old culture of the corresponding strain, and incubation was carried out at 28 ± 1 °C for 14 days [32]. The mycelium was separated from the culture broth and dried to constant mass at 105 ± 5 °C [33]. The culture filtrate was used to determine pectinesterase and polygalacturonase activities.

Methods for determining pectinase activity

Pectinesterase activity (EC 3.1.1.11) was determined titrimetrically using a modified method of Dudka et al. [34] and Pednekar et al. [35]. The reaction mixture, consisting of the culture filtrate (as the enzyme source) and a 1% pectin solution, was incubated at 30 °C for 180 min. After incubation, the mixture was titrated with 0.05 N NaOH to pH 7.5. One unit of pectinesterase activity (PE, U) was defined as the amount of enzyme catalyzing the release of 1 μEq of acid per minute [35].

Exopolygalacturonase activity (EC 3.2.1.15) was determined based on the amount of reducing sugars released from pectin using the dinitrosalicylic acid (DNS) method with a glucose calibration curve, according to a modified method of Alajlani M. The reaction mixture, consisting of the culture filtrate and a 1% pectin solution, was incubated at 40 °C for 10 min. One unit of polygalacturonase activity (PG, U) was defined as the amount of enzyme catalyzing the formation of 1 μmol of reducing sugars per minute [36].

Specific enzyme activity was calculated using the previously determined biomass concentration in the culture broth according to the following formula:

$$SA = \frac{EA}{C_{BM}} ,$$

where EA is the activity of pectinolytic enzymes in the culture broth [37].

Statistical data processing

Two statistical methods were applied for data analysis: Student's *t*-test (for comparison of parameters of a single strain cultivated on two different media) and Duncan's multiple range test (for comparison among all strains within the same type of medium).

Results are presented as mean values ± standard deviation (SD) based on three independent measurements. Asterisks (*) indicate significant differences between results obtained on the two media at $P < 0.05$ according to Student's t-test, whereas different letters indicate significant differences among strains within each medium at $P < 0.05$ according to Duncan's test.

Results

At the first stage of the study, all *T. hirsuta* strains were cultivated on solid nutrient media, including a medium containing pectin as the sole carbon source (PYPA). The growth dynamics on these media are presented in Fig. 1. All strains colonized the Petri dishes within less than 10 days. The linear growth phase of all examined strains began on day 2 of the experiment.

Trametes hirsuta strains 338 and 359 completely overgrew the surface of the Petri dishes by day 9. Strain *T. hirsuta* 1569 reached full surface colonization by day 5. Strains *T. hirsuta* 5018 and 5137 completed colonization by day 7. The remaining strains overgrew the entire surface of the medium within a comparable cultivation period.

The obtained data on growth dynamics were used to calculate the growth rates of the investigated *T. hirsuta* strains. The results of these calculations are presented in Fig. 2.

The data presented in Fig. 2 indicate statistically significant differences in the radial growth rate (RGR) of the investigated *T. hirsuta* strains cultivated on pectin-containing medium (PYPA). RGR values varied among strains, reflecting strain-specific differences in pectin utilization. The lowest RGR on PYPA was observed for *T. hirsuta* 338, whereas *T. hirsuta* 1569 exhibited the highest growth rate, reaching 10.25 ± 0.46 mm/day. Strains *T. hirsuta* 5018 and 5137 demonstrated intermediate RGR values on PYPA, ranging from 6.43 to 7.11 mm/day. Overall, the observed variability in RGR highlights differences in the efficiency of radial growth among *T. hirsuta* strains on pectin-based medium.

The next step was the determination of the pectinase activity index. The results are presented in Fig. 3.

As shown in Fig. 3, the average pectinase activity index (PAI) for *T. hirsuta* was 1.00 ± 0.05. Among the studied strains, three exhibited above-average PAI values (*T. hirsuta* 338, 359, and 1569). PAI values exceeding 1.00 indicate that the diameter of the halo zone after treatment with 1% cetyltrimethylammonium bromide (CTAB) exceeds the diameter of the mycelial colony. The highest PAI was recorded for *T. hirsuta*

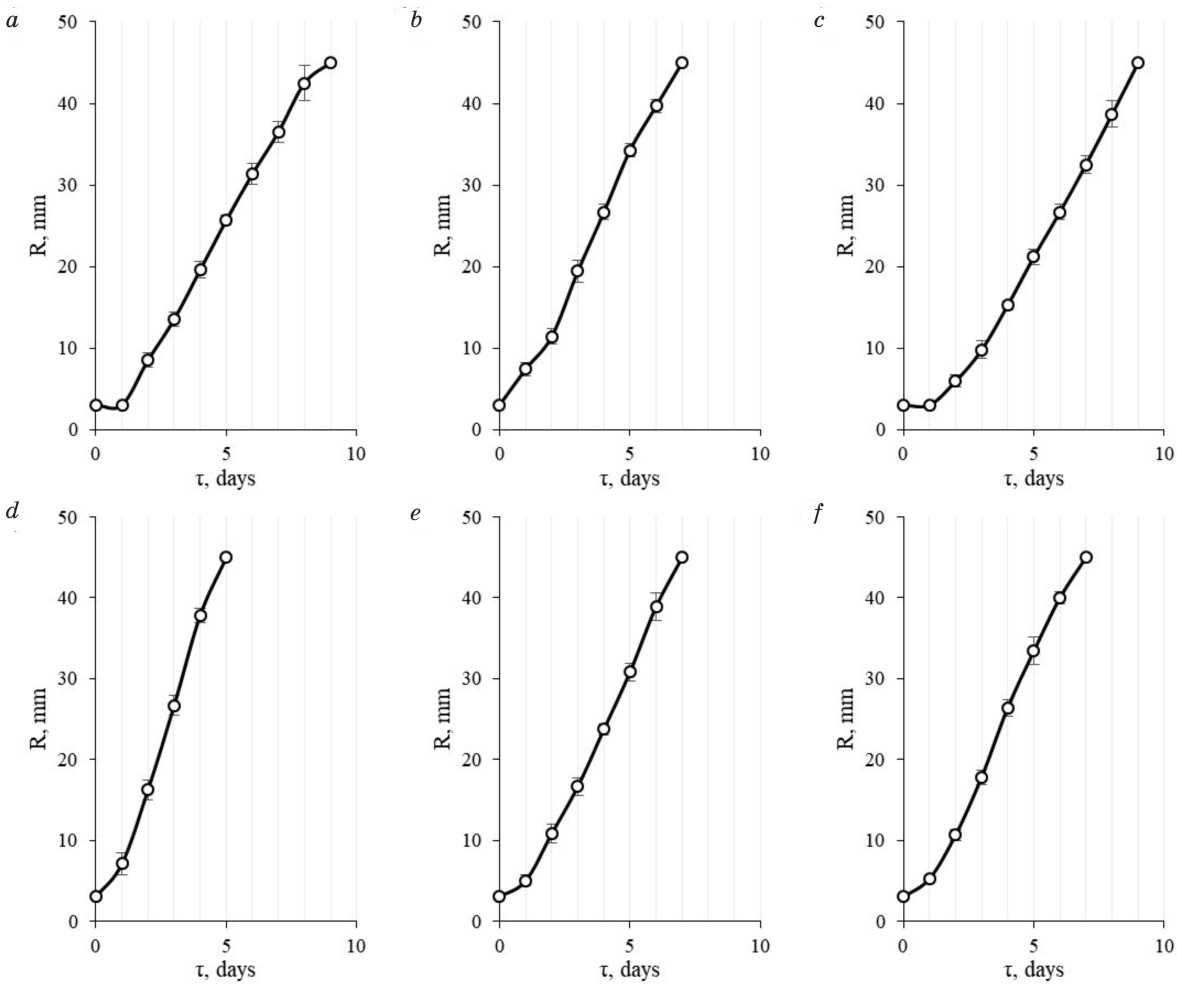

Fig. 1. **Growth dynamics of the investigated *T. hirsuta* strains:**
a — 338; *b* — 358; *c* — 359; *d* —1569; *e* — 5018; *f* — 5019

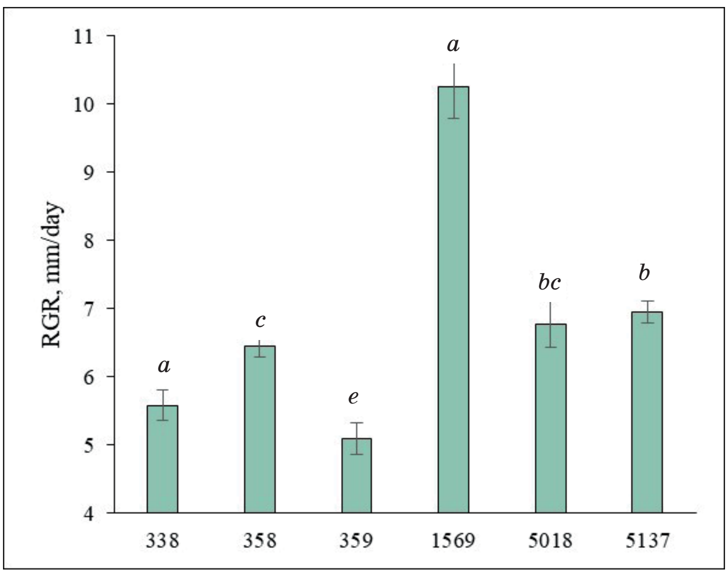

Fig. 2. **Radial growth rate of *T. hirsuta* strains on agar medium**
Note. Different letters indicate significant differences among strains within each medium at $P < 0.05$ according to Duncan's test

1569 (1.08 ± 0.04), which is 8% higher than the mean PAI value, indicating the greatest potential of this strain for pectinolytic enzyme production. Similar PAI values were observed for *T. hirsuta* 338 and 359 (1.02 ± 0.02 and 1.04 ± 0.02, respectively). In contrast, *T. hirsuta* 358, 5018, and 5137 showed comparable lower PAI values (0.97 ± 0.07), which are approximately 10% lower than that of *T. hirsuta* 1569.

Therefore, strains with the lowest PAI values were excluded from further experiments.

At the second stage of the study, strains *T. hirsuta* 338, 359, and 1569 were cultivated under static conditions in a liquid nutrient medium, and pectinolytic enzyme activity in the culture filtrate was determined. The strains were compared in terms of their pectinase activity and enzyme production capacity. The obtained results are presented in Fig. 4 and Table 1.

T. hirsuta 338 exhibited the lowest activity values for all investigated enzymes compared with the other strains (Fig. 4). Strains *T. hirsuta* 359 and 1569 demonstrated

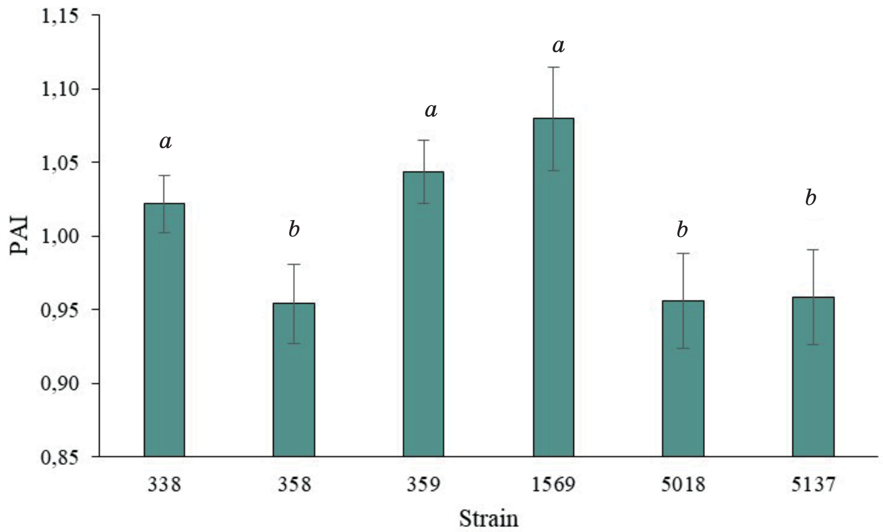

Fig. 3. **Pectinase activity index of *T. hirsuta* strains on agarized media**
Note. Different letters indicate significant differences among strains within each medium at $P < 0.05$ according to Duncan's test

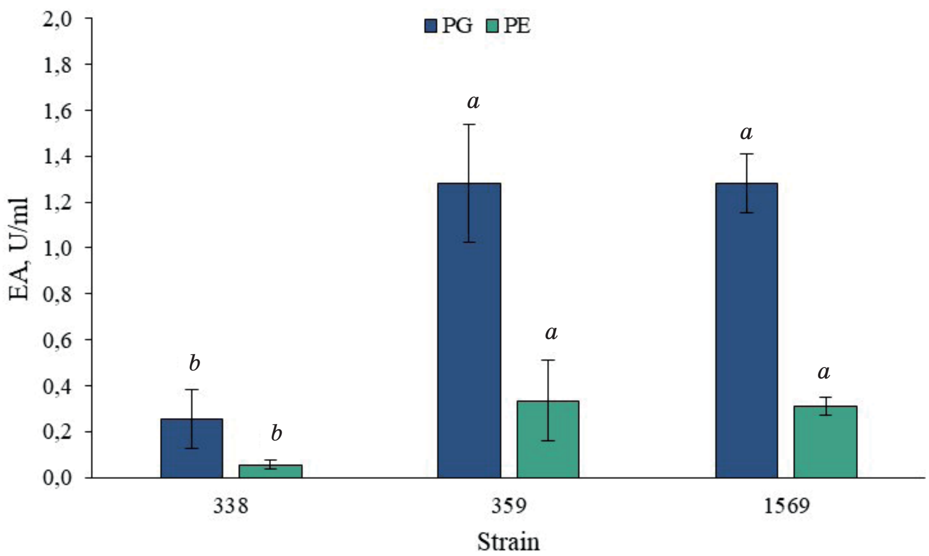

Fig. 4. **Pectinolytic enzyme activity of *T. hirsuta* strains**
Note. Different letters indicate significant differences among strains within each medium at $P < 0.05$ according to Duncan's test

Table 1. **Productivity of pectinolytic enzyme synthesis by *T. hirsuta* strains**

Strain	Productivity, U/gDW	
	PG	PE
T. hirsuta 338	45.35 ± 24.91[c]	9.77 ± 3.38[b]
T. hirsuta 359	182.30 ± 34.97[b]	47.37 ± 24.53[a]
T. hirsuta 1569	276.91 ± 57.86[a]	67.03 ± 14.25[a]

Note. Different letters indicate significant differences among strains within each medium at $P < 0.05$ according to Duncan's test

comparable polygalacturonase activity (0.25–1.28 U/mL), which was more than fivefold higher than that of *T. hirsuta* 338. These same strains also showed similar pectinesterase activity, with values approximately sixfold higher than those of *T. hirsuta* 338: 0.33 ± 0.18 U/mL and 0.31 ± 0.04 U/mL for *T. hirsuta* 359 and 1569, respectively.

From Table 1, it is evident that *T. hirsuta* 1569 exhibited the highest productivity of pectinolytic enzyme synthesis. Polygalacturonase production by *T. hirsuta* 1569 was sixfold higher than that of *T. hirsuta* 338 and 50% higher compared with *T. hirsuta* 359. The pectinesterase productivity of *T. hirsuta* 359 and 1569 did not differ significantly, although the mean value for the latter was 40% higher.

Discussion

This study characterized the enzymatic potential of *T. hirsuta* with respect to pectinolytic enzymes.

Cultivation of *T. hirsuta* strains on agar media containing glucose or pectin revealed differences in growth rates, confirming the influence of the carbon source on mycelial development. It was found that *T. hirsuta* 338 and 359 exhibited higher growth rates on glucose-based media, whereas *T. hirsuta* 5018 and 5137 grew better on pectin-containing media. These findings are consistent with previous studies showing that fungal strains possess distinct metabolic adaptations to specific carbon sources. Benoit et al. reported that the growth of various fungal species, including *Phanerochaete chrysosporium*, on pectin-containing media is accompanied by the induction of pectinolytic enzymes, which represents a typical biological response to the presence of an inducing substrate [38]. The action of pectinolytic enzymes in the medium leads to the release of pectin monomers [39], which may exert either positive [40] or negative effects on fungal growth [41].

The composition of the nutrient medium significantly affects the radial growth rate of basidiomycetous macromycetes, as supported by the results of this study [42]. Cultivation of fungi on media containing polysaccharides is generally accompanied by a reduction in growth rate. Similar results were reported by Pasailiuk et al. for *Hericium coralloides*, *Schizophyllum commune*, and *Fomes fomentarius* cultivated on Czapek medium with cellulose as the sole carbon source; all investigated strains exhibited a substantial decrease in growth rate. The authors observed a 3–30-fold reduction in colony growth rates. The largest difference was recorded for *Hericium coralloides* 2332 compared with agarized beer wort, indicating that cellulose is an unsuitable carbon source for its cultivation [43].

Our previous study indicated that high radial growth rates on pectin-based media do not necessarily correlate with elevated pectinolytic enzyme synthesis. In *T. ochracea*, moderate correlations between growth and pectinase activity were observed and were dependent on pH, whereas *T. versicolor* strains showed no clear relationship. In the present study, a similar trend was generally observed for *T. hirsuta*, as rapid growth was not uniformly accompanied by high pectinase activity across all strains [44].

The obtained data suggest that pectin-containing media may be used for macromycete cultivation when appropriate. Antonenko et al. investigated the growth of several macromycete strains used in this study on various nutrient media. The radial growth rate values recorded for *T. hirsuta* 338 and *T. hirsuta* 359 on pectin-containing medium were lower than those reported for all tested media, except for *T. hirsuta* 359 grown on agarized beer wort. For the other strains, growth rate values on pectin-containing medium were higher than or comparable to literature data. The largest difference was observed for *T. hirsuta* 1569, exceeding 2.5-fold [45]. Overall, the reduction in radial growth rate on medium containing a pectinase inducer (in this case, pectin) may be associated with additional energy costs required for the biosynthesis of pectinolytic enzymes, which may indicate active pectinase producers [46].

Analysis of pectinase activity in surface culture revealed pronounced intraspecific variability. Half of the investigated strains exhibited PAI values below the calculated mean (< 1.00 ± 0.05), whereas the remaining strains showed above-average values. Saroj et al. analyzed cellulase and xylanase activities using a similar approach based on an enzymatic activity index. In their study, the indices for cellulase and xylanase were identical (1.03 ± 0.10 and 1.03 ± 0.00, respectively) [47]. The mean PAI values obtained in the present study for three *T. hirsuta* strains were slightly lower than those reported for cellulase and xylanase activity in *P. pulmonarius*. Only *T. hirsuta* 338, 359, and 1569 showed values close to those reported for *P. pulmonarius* (1.02–1.08). *T. hirsuta* 1569 differed from most previously described *Trametes* strains by combining the highest radial growth rate with the highest pectinase activity index (PAI = 1.08 ± 0.04), which is comparable to or slightly higher than the maximum PAI values reported for *T. versicolor* [44].

Strains exhibiting higher pectinase activity index values may represent more efficient enzyme producers [48]. Similar tendencies were observed in the present study. Among the strains selected for submerged cultivation, an increase in pectinolytic enzyme production was observed in the sequence *T. hirsuta* 338 → 359 → 1569, corresponding to their PAI values in surface culture.

The choice of carbon source is considered an important factor in pectinase research, as its qualitative and quantitative characteristics significantly influence enzyme synthesis [49]. The presence of glucose, particularly as the sole carbon source, may suppress pectinase biosynthesis [50, 51]. However, contradictory findings have also been reported. Singh and Mandal demonstrated higher yields of polygalacturonase and pectate trans-eliminase on glucose, whereas pectin lyase production was enhanced in the presence of pectin [41]. In the present study, a standard glucose–peptone–yeast medium was used to evaluate pectinolytic activity. *T. hirsuta* 359 showed low total pectinase activity, which may indicate repression of the corresponding genes under the applied conditions. In contrast, the other strains did not exhibit such repression, suggesting constitutive synthesis of the studied pectinases.

The investigated *T. hirsuta* strains demonstrated pronounced strain-specific differences in pectinolytic enzyme activity, similar to those reported for *T. versicolor*. *T. hirsuta* 338 exhibited the lowest polygalacturonase and pectinesterase activities, whereas strains 359 and 1569 showed substantially higher values. Notably, *T. hirsuta* 1569 combined the highest growth rate and enzyme productivity, with polygalacturonase activity sixfold higher than that of strain 338 and 50% higher than that of 359, paralleling the dominant enzymatic performance observed in *T. versicolor* strains. These results confirm that, within the genus *Trametes*, strain-specific characteristics are more decisive for pectinolytic potential than species-level affiliation, highlighting the importance of targeted strain selection for pectinase production [52].

Similarly to the approach used in this study, Poveda et al. applied a comparable strategy for screening enzyme producers among micromycetes isolated from Antarctic marine sponges. After selecting the most active strain in surface culture based on an enzymatic activity index, the authors proceeded with submerged cultivation. The highest activity recorded for *Geomyces* sp. F09-T3-2 was 121 U/mg protein [31].

Martínez-Trujillo et al. reported a maximum polygalacturonase productivity of 0.52 U/mg dry biomass for *Aspergillus flavipes* FP-500 [53]. In the present study, the highest value reached 0.28 ± 0.11 U/mg dry biomass, indicating that micromycetes may exhibit higher polygalacturonase productivity than macromycetes under comparable conditions. However, Ketipally et al. reported a much lower productivity (0.04 U/mg dry biomass) for *A. nomius* MR103, which is approximately sevenfold lower than that of *T. hirsuta* 1569 but comparable to *T. hirsuta* 338 [54].

Conclusions

The study investigated the growth dynamics, radial growth rate, and pectinolytic enzyme activity of *T. hirsuta* strains cultivated on different media. The highest radial growth rate on both media was recorded for *T. hirsuta* 1569 (10.25 ± 0.46 mm/day on pectin-containing agar medium). Strains *T. hirsuta* 338, 359, and 1569 exhibited the highest pectinase activity in surface culture, with an average value of 1.05 ± 0.04.

T. hirsuta 359 and 1569 demonstrated the highest polygalacturonase and pectinesterase activities. Polygalacturonase activity was 1.28 ± 0.26 U/mL for *T. hirsuta* 359 and 1.28 ± 0.13 U/mL for *T. hirsuta* 1569, while pectinesterase activity was 0.33 ± 0.18 U/mL and 0.31 ± 0.04 U/mL, respectively. Pectinesterase productivity did not differ significantly between these two strains.

The obtained results indicate that *T. hirsuta* 1569 is the most promising strain for application in industrial processes aimed at the utilization of pectin-containing substrates.

Author contributions

Z.R.P.: conceptualization, investigation, data analysis, writing–original draft preparation; D.L.P.: writing—review and editing; K.I.R.: conceptualization, project administration, writing–review and editing. All authors contributed to manuscript revision and approved the submitted version.

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Conflict of interest

The authors declare no conflict of interest.

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ПЕКТОЛІТИЧНА АКТИВНІСТЬ МАКРОМІЦЕТІВ *Trametes hirsuta* (Wulfen) Lloyd У ПОВЕРХНЕВІЙ ТА ГЛИБИННІЙ КУЛЬТУРІ

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Пектинолітичні ензими мають значне промислове значення завдяки своїй здатності розкладати пектин у клітинній стінці рослин. Останнім часом все більша увага приділяється базидіоміцетам, зокрема роду *Trametes*, які здатні синтезувати складні ензиматичні системи; проте їхній потенціал для виробництва пектинази залишається недостатньо вивченим.

Мета. Вивчення росту та ензиматичної активності базидіоміцету *Trametes hirsuta* у поверхневій та глибинній культурах з метою розширення біотехнологічного потенціалу використання цих організмів як продуцентів пектиназ.

Матеріали й методи. Використано шість штамів макроміцету *Trametes hirsuta*, депонованих в Інституті ботаніки Національної академії наук України. Для культивування застосовували пептонно-дріжджове середовище з пектином (тверде поживне середовище). Ріст міцелію вимірювали щодня, а активність пектинази оцінювали за допомогою розчину цетриміду. Глибинне культивування проводили в глюкозно-пептонно-дріжджовому середовищі протягом 14 днів, після чого аналізували вміст потеїну, суху речовину та активність пектолітичних ензимів. Статистична обробка включала *t*-критерій Стьюдента та тест Дункана з використанням програмного забезпечення R та Excel.

Результати. Штами *T. hirsuta* 5018 та 5137 активніше розвивалися на пектині. Загалом, час надмірного росту коливався від 5 до 9 діб, залежно від штаму. Найвища швидкість радіального росту була зафіксована для штаму *T. hirsuta* 1569 (10,25 мм/добу). Було визначено індекс пектиназної активності (PAI), причому штам *T. hirsuta* 1569 показав найвищі значення (1,08 ± 0,04), що свідчить про його високий потенціал для синтезу пектинолітичних ферментів. Під час глибинного культивування штам *T. hirsuta* 1569 продемонстрував високу полігалактуроназну активність (1,28 ± 0,13 Од/мл) та пектинестеразну активність (0,31 ± 0,04 Од/мл). Натомість штам *T. hirsuta* 338 продемонстрував значно нижчі значення, що дозволило виключити його з подальших експериментів.

Висновки. Отримані результати свідчать про те, що штам *T. hirsuta* 1569 є найбільш перспективним для практичного застосування на основі його пектиназної активності. Тому його буде використано на наступних етапах дослідження для отримання пектинолітичного ензиматичного препарату.

Ключові слова: *Trametes*, пектинолітичні ензими, швидкість росту, пектиназна активність, полігалактуроназа, пектинестераза, біотехнологія.

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