

SCREENING OF CULTIVATION CONDITIONS FOR *TRAMETES HIRSUTA* IBK 1569 FOR ENDOPOLY GALACTURONASE PRODUCTION

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The study investigated the conditions for endopolygalacturonase synthesis by the *Trametes hirsuta* 1569 strain under submerged cultivation. It was established that the maximum enzyme activity was achieved on the 9th day, after which a decrease in activity was observed. Among the studied carbon sources, sugar beet pulp proved to be the most effective, whereas brewer's spent grain did not induce enzyme synthesis. The optimal concentration of sugar beet pulp was 10 g/dm³, which ensured a high level of activity without a further saturation effect. Maximum enzyme synthesis was observed when urea and ammonium sulfate were used as nitrogen sources, with the optimal concentration of (NH₄)₂SO₄ being 1.0 g/dm³. The most favorable pH value for endopolygalacturonase synthesis was in the range of 5–7, with a maximum at pH 6. A moderate agitation rate (120 rpm) and inoculum size of 5 % ensured maximum enzyme synthesis. The obtained results indicate the feasibility of using lignocellulosic wastes, particularly sugar beet pulp, for endopolygalacturonase biosynthesis and can be used to optimize biotechnological processes.

Keywords: biotechnology, pectinolytic enzymes, cultivation parameters, nutrient medium composition, enzyme yield.

Introduction. Pectinolytic enzymes (pectinases) are a group of enzymes that catalyze the hydrolysis of pectic substances, which are important structural components of plant cell walls and consist mainly of D-galacturonic acid residues linked by α -1,4-glycosidic bonds (Patel et al., 2024). This group includes polygalacturonases, pectin lyases, pectate lyases, and pectin esterases, which differ in their mechanism of action on the pectin molecule. Endopolygalacturonase (EC 3.2.1.15) is of particular industrial interest, as it catalyzes the hydrolysis of internal glycosidic bonds in the polygalacturonic chain, causing pectin depolymerization and a decrease in the viscosity of pectin solutions (Hao et al., 2022; Jayani et al., 2010; Sharma et al., 2024).

The main producers of pectinolytic enzymes are microorganisms, among which microscopic fungi play a leading role. Their advantage lies in their ability to secrete a significant portion of the synthesized enzymes into the culture liquid, which greatly simplifies their subsequent recovery (Alqahtani et al., 2022; Bassim Atta et al., 2022). Basidiomycete macromycetes of the genus *Trametes* are promising producers of extracellular enzymes capable of synthesizing a wide range of enzymes involved in the degradation of plant polymers (Giouroukou et al., 2026). In our previous studies, fungi of the genus *Trametes* demonstrated their potential as promising producers of pectinolytic enzymes. Among the studied species (*T. versicolor*, *T. ochracea*, *T. hirsuta*), the latter, namely *T. hirsuta*

1569, proved to be the most promising for further research (P. R. Zubyk et al., 2024).

It is known that the level of pectinase synthesis largely depends on cultivation conditions. Among the main factors affecting enzyme production by *Aspergillus* spp., *Bacillus* spp., *Streptomyces* sp., and others are the composition of the nutrient medium (type and concentration of carbon and nitrogen sources), medium pH, agitation rate (under submerged cultivation conditions), cultivation time, and others. Changes in these parameters can lead to significant fluctuations in enzyme synthesis levels; therefore, the selection of optimal cultivation conditions is one of the key stages in the development of efficient biotechnological processes for enzyme production (Fontana et al., 2012; Javaid Asad M, 2015; Jayani et al., 2010; Rozendo et al., 2024; Serrat et al., 2004; Shrestha et al., 2023). An important approach is the use of available plant substrates, particularly agro-industrial wastes, which can serve not only as inducers of pectinolytic enzyme synthesis but also reduce the cost of nutrient media, which is important for industrial enzyme production. There are studies demonstrating the prospects of using wastes for the industrial production of endopolygalacturonase by *Aspergillus* spp., *Bacillus licheniformis*, and other producers (Jahan et al., 2017; Larios et al., 1989). Studies devoted to endopolygalacturonase synthesis by basidiomycete macromycetes are scarce. In particular, little attention has been paid to the influence of cultivation conditions on

endopolygalacturonase synthesis in fungi of this group, including *Trametes hirsuta*, which determines the relevance of further research in this area.

Objective of the study: to investigate the influence of the main cultivation parameters on endopolygalacturonase synthesis by the fungus *Trametes hirsuta* 1569 in submerged culture.

Materials and Methods. *Object of study.* The study used the *Trametes hirsuta* 1569 strain obtained from the M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine (IBK) (Bisko et al., 2024). The culture was maintained on agarized barley-malt extract (8° Balling) at 4 °C.

Preparation of inoculum. The inoculum was cultivated in 250 cm³ flasks containing 50 cm³ of glucose-peptone-yeast medium according to (Bondaruk et al., 2025) at a temperature of (28 ± 2) °C for 7 days.

Basic cultivation conditions. The basal nutrient medium consisted of: pectin (Apfelpektin, Germany) – 10.0 g/dm³, NH₄NO₃ (supplied by SpheraSim, Ukraine) – 1.0 g/dm³, KH₂PO₄ (supplied by Khimlaborreaktiv, Ukraine) – 1.0 g/dm³, MgSO₄·7H₂O (supplied by Khimlaborreaktiv, Ukraine) – 0.5 g/dm³, FeSO₄·7H₂O (supplied by Himreagent, Ukraine) – 5.0 mg/dm³, ZnSO₄·7H₂O (supplied by Labormarket, Ukraine) – 4.4 mg/dm³, CaCl₂ (supplied by Khimlaborreaktiv, Ukraine) – 5.5 mg/dm³, initial pH 6.5. The medium was autoclaved at 121 °C for 15 min (Danylyak et al., 1989). Submerged cultivation was carried out in 250 cm³ Erlenmeyer flasks containing 50 cm³ of medium without agitation at 28 °C.

Selection of cultivation conditions. Cultivation conditions were selected sequentially using the one-factor-at-a-time (OFAT) method to maximize endopolygalacturonase yield (Oumer et al., 2018). At each stage, only one parameter was varied, while the other factors were maintained at levels determined as optimal in the previous stages of the experiment. The concentrations of salts KH₂PO₄, MgSO₄·7H₂O, FeSO₄·7H₂O, ZnSO₄·7H₂O, CaCl₂ and the cultivation temperature were kept at the initial level. The parameters were varied in the following order: cultivation time, carbon source, its concentration and particle size, nitrogen source, its concentration, initial pH level, agitation rate, and inoculum size.

Cultivation time. To determine the optimal cultivation time, the flasks were incubated in the basal nutrient medium under initial conditions for 24–504 h with a step of 48 h. Further cultivations were carried out for the optimal duration determined in this experiment.

Carbon source, its particle size, and concentration. The qualitative composition of the

carbon source was tested at a concentration of 1 % (w/v, dry weight). Agricultural and industrial wastes were evaluated as alternative carbon sources: sugar beet pulp (Sugar factory in Rokytno, Ukraine), grape and apple pomaces (Institute of Viticulture and Winemaking «Magarach» of the National Academy of Agrarian Sciences of Ukraine, Ukraine), pomegranate, mandarin and pomelo peels (obtained from pomegranate, mandarin and pomelo purchased at the local supermarket Fora), barley-rye and barley-wheat spent grain (obtained from a by-product of distillate production from The Institute of Food Resources of the National Academy of Agrarian Sciences of Ukraine, Ukraine). Peels and spend grains were dried at 60 °C. The wastes were ground to a particle size of 0–1 mm. The selected optimal carbon source was used for further studies.

The particle size of the selected carbon source was determined using the following fractions: 0–1 mm, 1–3 mm, 3–5 mm, 5–7 mm, and 7–10 mm. The determined optimal particle size of the carbon source was used in further studies.

The concentration of the selected carbon source was determined by cultivating the basidiomycete macromycete on a nutrient medium with different contents of this component: from 5 g/dm³ to 35 g/dm³ with a step of 5 g/dm³. The optimal concentration of the carbon source was used in further studies.

Nitrogen source and concentration. The qualitative composition of the nitrogen source was tested at a concentration of 1 % (w/v). In this study, the following substances were used: KNO₃ (supplied by SpheraSim, Ukraine), (NH₄)₂SO₄ (supplied by Labormarket, Ukraine), NH₄NO₃ (supplied by Labormarket, Ukraine), Ca(NO₃)₂ (supplied by Khimlaborreaktiv, Ukraine), casein (supplied by Kharkov Torg, Ukraine), urea (supplied by Khimlaborreaktiv, Ukraine), yeast extract (supplied by Khimlaborreaktiv, Ukraine), and peptone (supplied by Khimlaborreaktiv, Ukraine).

The concentration of the selected nitrogen source was determined by cultivating the basidiomycete macromycete on a nutrient medium with different contents of this component: from 0.5 g/dm³ to 3.0 g/dm³ with a step of 0.5 g/dm³. The optimal concentration of the nitrogen source was used in further studies.

Medium pH. The initial pH value of the nutrient medium after autoclaving was adjusted from 3.0 to 9.0 with a step of 1.0 using the optimal medium composition. The determined optimal pH value was used in further studies.

Agitation rate. The agitation rate was varied from 100 rpm to 200 rpm with a step of 20 rpm using an

orbital shaker. The determined optimal agitation rate was used in further studies.

Inoculum size. The inoculum was added in amounts of 1 %, 2.5 %, 5 %, 7.5 %, and 10 % (v/v). The optimal inoculum size was determined.

Determination of enzyme activity. Endopolygalacturonase activity (EndoPG, EC 3.2.1.171) was determined by the degree of pectin viscosity reduction using the method described in (Dudka et al., 1982). The reaction mixture containing culture liquid and a 0.5 % solution of apple pectin (pH 5.0) was incubated at 30 °C for 10 min in an Ostwald viscometer (d = 0.73 mm, Labexpert). One unit of endopolygalacturonase activity (EndoPGA, U) was defined as the amount of enzyme that reduces the viscosity of the pectin solution by 30 %.

After that, the relative endopolygalacturonase activity was calculated using the formula (Oumer et al., 2018):

$$relEndoPGA = \frac{EndoPGA}{\max(EndoPGA)} \cdot 100\%$$

Statistical analysis. Statistical analysis of the data was performed using Duncan's test. All experiments were carried out in triplicate, and the results are presented as $M \pm m$ (mean value \pm standard deviation) obtained from three measurements (n=3). The results were considered statistically significant at p-value < 0.05. Letters *a, b, c, d*, etc. indicate differences between strains: $p < 0.05$ according to Duncan's test.

Data processing and graph plotting were performed using Excel software (USA).

Results and Discussion. The dynamics of endopolygalacturonase synthesis by *Trametes hirsuta* 1569 were studied over 504 h of cultivation in the basal nutrient medium (Fig. 1).

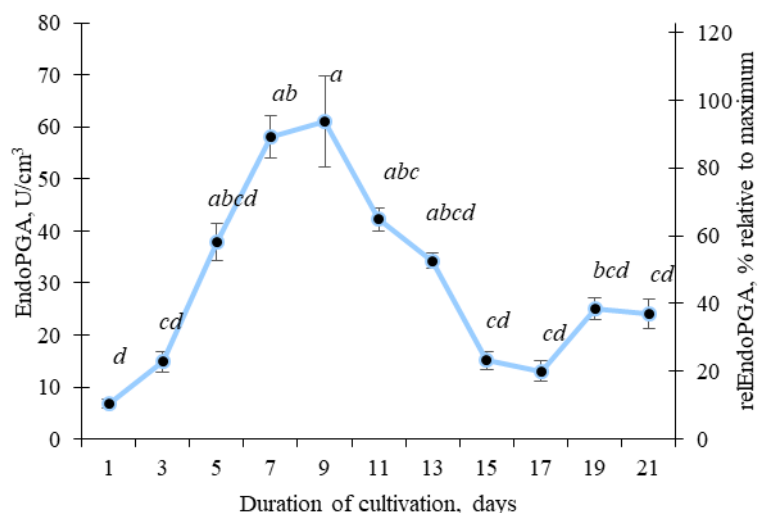


Fig. 1. Dynamics of endopolygalacturonase synthesis by *Trametes hirsuta* 1569

Enzyme activity gradually increased during mycelial growth and reached a maximum on the 9th day of cultivation (61.09 ± 8.68 U/cm³). This value did not differ significantly from that obtained on the 7th day (58.10 ± 4.02 U/cm³) according to Duncan's test ($p < 0.05$). Further extension of the cultivation period was accompanied by a decrease in enzyme activity, and after the 15th day it remained at a consistently low level ($p > 0.05$) until the end of the experiment. Based on the obtained results and unpublished data on our other enzymes of the pectinolytic complex, the cultivation time for further studies was set at 9 days.

The effects of different lignocellulosic wastes as carbon sources, its concentration and particle size on endopolygalacturonase synthesis by *Trametes hirsuta* 1569 are shown in Fig. 2).

The highest EndoPG activity (Fig. 2.A) was recorded when sugar beet pulp was used (29.38 ± 2.94 U/cm³), which was statistically higher than the values obtained for most of the tested substrates ($p < 0.05$). High activity values were also observed when mandarin peels (17.88 ± 1.94 U/cm³) and pomelo peels (16.77 ± 1.82 U/cm³) were used; however, they did not differ significantly from each other. Significantly lower levels of enzymatic activity were recorded when grape and apple pomace, corn bran, and pomegranate peels were used (10–40 % of the maximum value). At the same time, when brewer's spent grain (barley-rye and barley-wheat) was used, endopolygalacturonase synthesis practically did not occur.

The obtained results indicate that sugar beet pulp is the most effective inducer of endopolygalacturonase synthesis by *T. hirsuta* 1569

among the tested substrates; therefore, it was used as the carbon source in further experiments.

After determining the optimal carbon source, the optimal concentration of sugar beet pulp was determined (Fig. 2.B). It was established that an increase in substrate concentration was accompanied by an increase in EndoPGA enzymatic activity. The maximum EndoPGA value was recorded at a

concentration of 25 g/dm³ (30.65 ± 2.99 U/cm³). At lower concentrations, the activity was reduced; in particular, at 5 g/dm³ it was about 60% of the maximum value. At the same time, within the range of 10–35 g/dm³, the activity values did not differ significantly according to Duncan's test ($p < 0.05$).

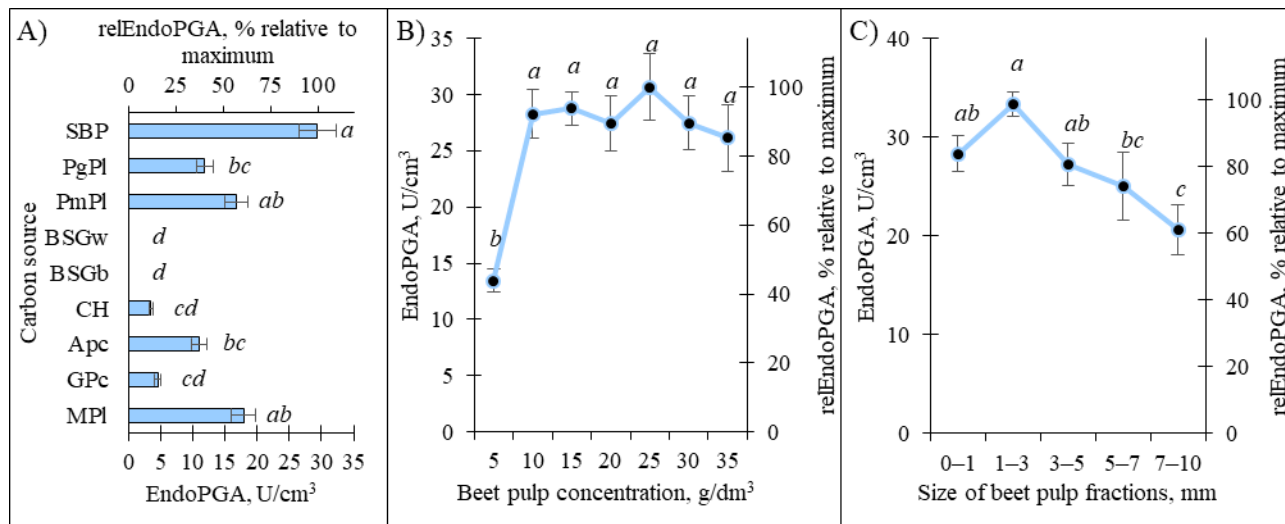


Fig. 2. Effect of carbon source type (A), its concentration (B) and particle size (C) on endopolygalacturonase synthesis by *Trametes hirsuta* 1569

Note: MPI – mandarin peels; GpC – grape pomace; Apc – apple pomace; CH – corn husk; BSGb – brewer's spent grain (barley); BSGw – brewer's spent grain (wheat); PmPI – pomelo peels; PgPI – pomegranate peels; SBP – sugar beet pulp

A further increase in substrate concentration above 10 g/dm³ did not lead to a significant increase in enzyme activity. Taking these results into account, a sugar beet pulp concentration of 10 g/dm³ was selected as optimal for further studies.

The enzymatic activity of *Trametes hirsuta* 1569 depended on the particle size of the substrate (Fig. 2.C). The maximum EndoPGA value was recorded when particles of 1–3 mm were used (33.26 ± 1.22 U/cm³). The activity values for the 3–5 mm fraction did not differ significantly according to Duncan's test ($p < 0.05$), although the mean EndoPGA value was about 15 % lower.

When smaller (0–1 mm) or larger substrate fractions (5–10 mm) were used, a decrease in enzymatic activity of approximately 20–35 % was observed. The obtained results indicate that the use of sugar beet pulp with a particle size of 1–3 mm provides favorable conditions for endopolygalacturonase synthesis by *T. hirsuta* 1569; therefore, this fraction was selected for further studies.

It was established that the level of enzymatic activity strongly depended on the nitrogen source (Fig. 3.A). The highest EndoPGA activity was recorded when urea was used (34.71 ± 1.48 U/cm³).

High activity values were also observed when ammonium salts, calcium nitrate, and peptone were used (77–95 % of the maximum value, $p < 0.05$ according to Duncan's test).

Lower levels of enzymatic activity were recorded when organic nitrogen sources – casein and yeast extract – were used (about 60 % of the maximum value). The lowest level of endopolygalacturonase synthesis was observed when KNO₃ was used (2.70 ± 0.26 U/cm³).

Although the obtained results indicate that urea is the most effective nitrogen source for endopolygalacturonase synthesis by *T. hirsuta* 1569, ammonium sulfate was selected for further studies, taking into account economic considerations, the absence of statistically significant differences between these sources, and our unpublished data on other enzymes of the pectinolytic complex.

The next step was to determine the optimal concentration of this salt (Fig. 3.B). The maximum endopolygalacturonase activity was recorded at an ammonium sulfate concentration of 1.0 g/dm³ (31.88 ± 5.21 U/cm³), which was statistically higher than the values obtained at lower (0.5 g/dm³) and higher (1.5–3.0 g/dm³) concentrations ($p < 0.05$).

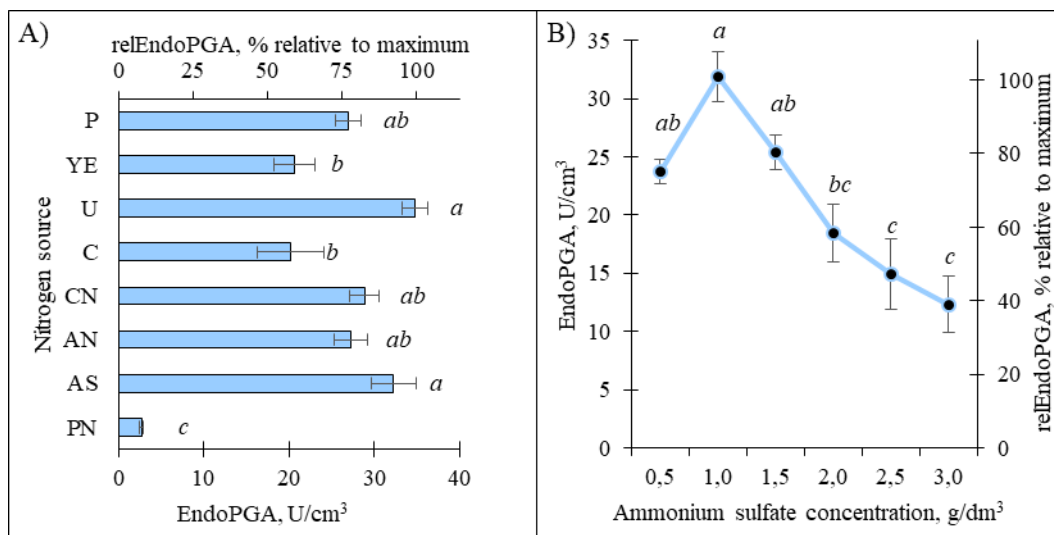


Fig. 3. Effect of nitrogen source type (A) and its concentration (B) on endopolygalacturonase synthesis by *Trametes hirsuta* 1569

Note: PN – potassium nitrate; AS – ammonium sulfate; AN – ammonium nitrate; CN – calcium nitrate; K – casein; U – urea; YE – yeast extract; P – peptone

A sharp decrease in EndoPG activity was observed with increasing nitrogen content in the nutrient medium. Thus, the optimal concentration of the selected nitrogen source for enzyme synthesis was 1.0 g/dm³. The next step was to determine the optimal concentration of this salt (Fig. 3.B). The maximum endopolygalacturonase activity was recorded at an ammonium sulfate concentration of 1.0 g/dm³ (31.88 ± 5.21 U/cm³), which was statistically higher than the values obtained at lower (0.5 g/dm³) and higher (1.5–3.0 g/dm³) concentrations (p < 0.05). A sharp decrease in

EndoPG activity was observed with increasing nitrogen content in the nutrient medium. Thus, the optimal concentration of the selected nitrogen source for enzyme synthesis was 1.0 g/dm³.

The maximum enzymatic activity was recorded at pH 6 (27.96 ± 2.10 U/cm³), which was statistically higher than the values obtained at more acidic (pH 3–4) and alkaline (pH 8–9) values by 25–75% (Fig. 4.A). Within the pH range of 5–7, high activity levels were observed that did not differ significantly according to Duncan's test (p < 0.05).

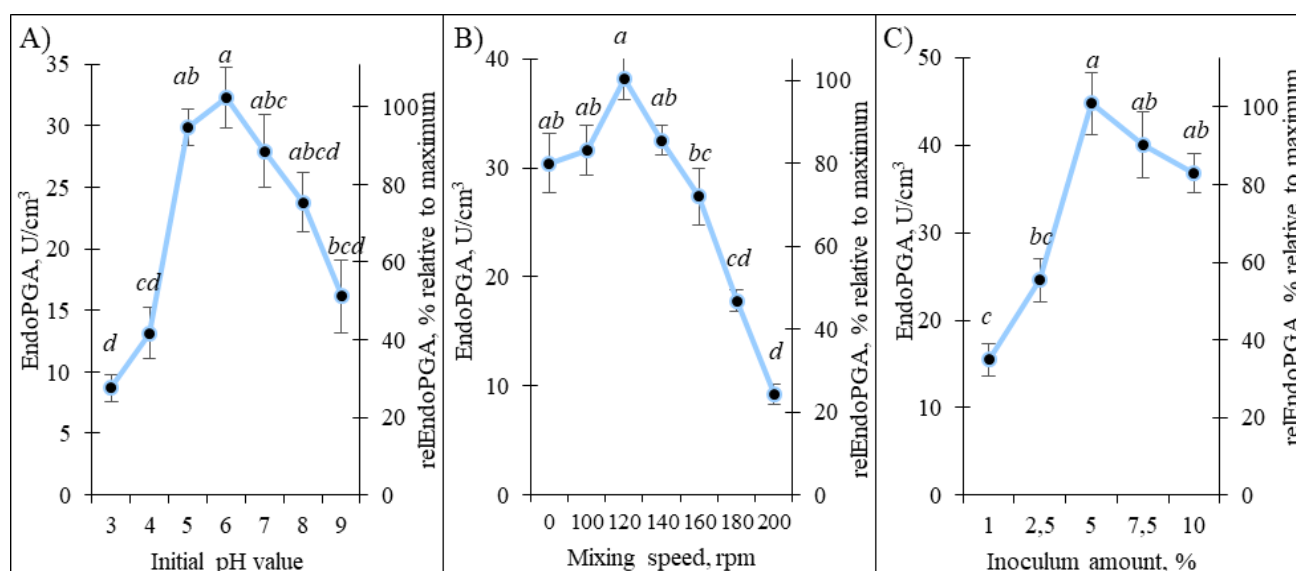


Fig. 4. Effects of initial pH (A), agitation rate (B) and inoculum size (C) on endopolygalacturonase synthesis by *Trametes hirsuta* 1569

The obtained results indicate that a slightly acidic to neutral pH range is optimal for endopolygalacturonase synthesis, with a maximum at pH 6, which was selected for further studies.

The maximum enzymatic activity (38.16 ± 1.87 U/cm³) was observed at 120 rpm (Fig. 4.B); however, within the range of 0–140 rpm the values did not differ significantly (p < 0.05). Further

increases in agitation rate were accompanied by a decrease in activity by 30–85 %, and at 180–200 rpm statistically lower values were recorded, with a minimum at 200 rpm. The obtained results indicate that a moderate agitation rate is optimal for endopolygalacturonase synthesis, whereas excessive agitation negatively affects enzyme production. Therefore, for further studies, *T. hirsuta* 1569 was cultivated at 120 rpm.

Endopolygalacturonase activity depended on the inoculum size (Fig. 4.C). The maximum enzymatic activity was observed at an inoculum size of 5 % (44.73 ± 3.59 U/cm³), which was statistically higher than the values obtained at lower inoculum volumes ($p < 0.05$). Within the range of 5–10 %, the activity remained at a high level and did not differ significantly between individual variants. When the inoculum size was reduced to 1–2.5 %, a significant decrease in enzyme synthesis was observed, by approximately 45–65 %. The obtained results indicate that the optimal inoculum size for further studies is 5 %.

The dynamics of endopolygalacturonase synthesis by *Trametes hirsuta* 1569 are characterized by a rapid increase in activity during the first 7–9 days with a peak on the 9th day, followed by a decrease after 15 days, which is associated with the transition to the stationary phase, mycelial autolysis, and degradation of proteins in the culture liquid, including enzymes (Liu et al., 2014; Vasina et al., 2016). A similar trend is observed for other fungal pectinases; however, the absolute time values may differ significantly depending on the organism studied. In particular, for *Lentinus tigrinus*, the maximum polygalacturonase production was achieved on the 9th day of cultivation, which is comparable with the obtained results (dos Santos et al., 2024). At the same time, for other fungi, for example *Piriformospora indica*, the maximum activity was observed already on the 6th day, that is, earlier than in the present study (Heidarizadeh et al., 2018). Such differences may be caused by variations in growth rate or regulation of enzyme synthesis and are likely to be strain-specific for *T. hirsuta* 1569.

The obtained results regarding the effect of the carbon source showed the advantage of sugar beet pulp as an inducer of enzyme synthesis. In general, this is consistent with data indicating that pectin-containing agro-industrial wastes (for example, citrus or fruit residues) effectively stimulate polygalacturonase production in fungi (Samreen et al., 2019). In a study with *Thermoascus aurantiacus*, the highest enzyme production was also observed when juice-processing wastes were used as a substrate (Martins et al., 2012). At the same time, the literature notes that the effectiveness of different

wastes may vary significantly depending on their chemical composition, particularly the pectin content and associated components (Bevilaqua et al., 2026; P. Zubyk et al., 2025). This may explain why in the present study some substrates (for example, grape pomace, pomegranate peels, etc.) showed lower activity, while brewer's spent grain did not induce endopolygalacturonase synthesis regardless of its composition. Grape and apple pomace, pomegranate, pomelo, and mandarin peels contain higher amounts of phenolic compounds, which likely inhibit the accumulation of pectinolytic enzymes due to their effect on biomass yield (P. Zubyk et al., 2025).

The dependence of enzyme activity on substrate concentration in this study showed a saturation pattern. After reaching a sugar beet pulp concentration of 10 g/dm³, further increases in its amount did not lead to a significant increase in activity. A similar trend has been described in the literature, where excessively high concentrations of the carbon source may lead to inhibition or inefficient substrate utilization (Fontana et al., 2012). In *Aspergillus niger*, during optimization of submerged cultivation for pectinase production, the activity peak was observed in the inducer range of 30–35 g/L, after which repression of enzyme synthesis also occurred (El Enshasy et al., 2018).

It is known that particle size determines the surface area-to-volume ratio, substrate porosity, and the efficiency of mass and heat transfer, which directly affects mycelial growth and enzyme secretion (Bocchini Martins et al., 2011; Patidar et al., 2018). On the one hand, reducing particle size increases substrate availability for enzyme action due to the increased specific surface area, which promotes intensification of metabolic processes. However, excessive grinding leads to substrate compaction, decreased porosity, and reduced aeration, which limits oxygen diffusion and metabolic heat removal, resulting in decreased enzyme production (Ibrahim et al., 2013; Singh nee' Nigam et al., 2009). On the other hand, increasing particle size is accompanied by a decrease in specific surface area and limited access of microorganisms to nutrients, which also negatively affects enzyme activity (Alcantara et al., 2013).

Regarding the nitrogen source, it was found that inorganic compounds provided a higher level of enzyme synthesis compared to some organic sources. The literature also emphasizes that the composition of nitrogen nutrition significantly affects pectinase production; however, no universal pattern exists, and different strains may respond differently to organic and inorganic sources (Mathew et al., 2008).

The effect of pH showed that the optimum for endopolygalacturonase synthesis lies in the slightly acidic range. The literature typically reports pH optima for polygalacturonases in the range of approximately 3–7, with a maximum in the acidic region (Anand et al., 2017; Shrestha et al., 2023). For example, in *Aspergillus niger*, the optimum activity is around pH 4 (Bennamoun et al., 2016). At the same time, in some cases more neutral values have been reported: for the pectinolytic extract of *Aspergillus brasiliensis*, maximum activity was observed at pH 7, while activity remained high in the range of pH 5–6 (Falcão et al., 2024).

The effect of agitation rate demonstrated the presence of an optimum at moderate values and a sharp decrease in activity when this value was exceeded. A similar trend was observed in studies of submerged cultivation of fungi, where the optimal agitation rates are usually within the range of 100–150 rpm, whereas an increase to 180–200 rpm leads to a decrease in enzyme production. This may be associated with mechanical damage to the mycelium or changes in growth morphology (Ravichandran et al., 2022; Zhou et al., 2012).

Conclusions. As a result of the study, it was established that the synthesis of endopolygalacturonase by the strain *Trametes hirsuta* 1569 reaches its maximum on the 9th day of cultivation (~61 U/cm³), after which a decrease in activity is observed, indicating the impracticality of prolonged

cultivation under these conditions. Among the studied carbon sources, sugar beet pulp provided the highest level of enzymatic activity (29–30 U/cm³), whereas other substrates were characterized by significantly lower values. An increase in substrate concentration was accompanied by an increase in activity only up to 10 g/dm³, after which a saturation effect was observed. The most favorable fraction of sugar beet pulp for maximizing endopolygalacturonase yield was the 1–3 mm particle size fraction.

It was established that the nitrogen source significantly affects enzyme production: the highest values were obtained when urea and ammonium salts were used. The optimal concentration of (NH₄)₂SO₄ is 1.0 g/dm³, while deviations from this level lead to a decrease in activity.

Optimal cultivation parameters include pH 6, an agitation rate of 120 rpm, and inoculation with 5 % seed culture, at which the maximum synthesis level is achieved (~38–45 U/cm³).

The obtained results indicate the potential of endopolygalacturonase from *T. hirsuta* 1569 for scale-up and further studies on optimization of the nutrient medium composition.

Conflict of interest. The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

References:

- Alcantara, S. R., Leite, N. J., & da Silva, F. L. H. (2013). Scale up of polygalacturonase production by solid state fermentation process. In Food Industry (pp. 399–420). InTech. <https://doi.org/10.5772/53152>
- Alqahtani, Y. S., More, S. S., R., K., Shaikh, I. A., K. J., A., More, V. S., Niyonzima, F. N., Muddapur, U. M., & Khan, A. A. (2022). Production and purification of pectinase from *Bacillus subtilis* 15A-B92 and its biotechnological applications. *Molecules*, 27(13), 4195. <https://doi.org/10.3390/molecules27134195>
- Anand, G., Yadav, S., & Yadav, D. (2017). Production, purification and biochemical characterization of an exo-polygalacturonase from *Aspergillus niger* MTCC 478 suitable for clarification of orange juice. *3 Biotech*, 7(2), 122. <https://doi.org/10.1007/s13205-017-0760-3>
- Bassim Atta, M., & Ruiz-Larrea, F. (2022). Fungal pectinases in food technology. In Pectins - The New-Old Polysaccharides. IntechOpen. <https://doi.org/10.5772/intechopen.100910>
- Bennamoun, L., Hiligsmann, S., Dakhmouche, S., Ait-Kaki, A., Labbani, F.-Z., Nouadri, T., Meraihi, Z., Turchetti, B., Buzzini, P., & Thonart, P. (2016). Production and properties of a thermostable, pH-stable exo-polygalacturonase using *Aureobasidium pullulans* isolated from Saharan soil of Algeria grown on tomato pomace. *Foods*, 5(4), 72. <https://doi.org/10.3390/foods5040072>
- Bevilaqua, G. C., Gonçalves, I. S., & Forte, M. B. S. (2026). Comparison of pretreatment strategies for the integrated production of pectin and fermentable sugars from cocoa pod husk within a biorefinery approach. *Biomass and Bioenergy*, 212, 109245. <https://doi.org/10.1016/j.biombioe.2026.109245>
- Bisko, N., Lomberg, M., Mykchaylova, O., & Mytropolska, N. (2024). *IBK Mushroom Culture Collection. Version 1.8. The IBK Mushroom Culture Collection of the M.G. Kholodny Institute of Botany.* The IBK Mushroom Culture Collection of the M.G. Kholodny Institute of Botany. <https://doi.org/https://doi.org/10.15468/dzdsqu>
- Bocchini Martins, D. A., do Prado, H. F. A., Ribeiro Leite, R. S., Ferreira, H., Souza Moretti, M. M. de, Da, R., & Gomes, E. (2011). Agroindustrial wastes as substrates for microbial enzymes production and source of sugar for bioethanol production. In Integrated Waste Management - Volume II. InTech. <https://doi.org/10.5772/23377>
- Bondaruk, S. V., Bulava, S. O., Korzh, R. A., Lesyk, D. S., Polovynko, V. V., Fedyk, A. V., & Al-Maali, G. A. (2025). Biotransformation of 2,6-dichloroaniline and 3,5-dichloroaniline by the mycelium of basidiomycetes. *Ukrainian Botanical Journal*, 82(6), 594–603. <https://doi.org/10.15407/ukrbotj82.06.594>
- Danylyak, N. I., Semichaevsky, V. D., Dudchenko, L. G., & Trutneva, I. A. (1989). *Enzyme systems of*

- higher basidiomycetes (in russian: *Fermentnyye sistemy vysshikh bazidiomitsetov*). Kyiv: Naukova Dumka.
11. dos Santos, E. G., Assis, S. A. de, Ferreira, A. N., de Almeida Bezerra, M., de Paula, S. A., Valasques, L. M., do Nascimento Junior, B. B., & Lima Valasques Júnior, G. (2024). Production, characterization and application of polygalacturonase produced by *Lentinus tigrinus* CCMB 553. *Biocatalysis and Agricultural Biotechnology*, 58, 103216. <https://doi.org/10.1016/j.bcab.2024.103216>
 12. Dudka, I. A., Wasser, S. P., Ellanskaia, I. A., et al. (1982). *Methods of experimental mycology (in russian: Metody eksperimental'noy mikologii)* (V. I. Bilai (Ed.)). Kyiv: Naukova Dumka.
 13. El Enshasy, H. A., Elsayed, E. A., Suhaimi, N., Malek, R. A., & Esawy, M. (2018). Bioprocess optimization for pectinase production using *Aspergillus niger* in a submerged cultivation system. *BMC Biotechnology*, 18(1), 71. <https://doi.org/10.1186/s12896-018-0481-7>
 14. Falcão, L. de S., Monteiro, T. E. de A., do Amaral, T. S., Azevedo, S. C. M., Batista, B. N., Jordão, A. M., & Albuquerque, P. M. (2024). Optimized production of fungal polygalacturonase using cupuaçu (*Theobroma grandiflorum*) peel as substrate and its effect on clarification of cupuaçu juice. *Beverages*, 11(1), 6. <https://doi.org/10.3390/beverages11010006>
 15. Fontana, R. C., & Silveira, M. M. (2012). Influence of pectin, glucose, and pH on the production of endo- and exo-polygalacturonase by *Aspergillus oryzae* in liquid medium. *Brazilian Journal of Chemical Engineering*, 29(4), 683–690. <https://doi.org/10.1590/S0104-66322012000400001>
 16. Giouroukou, E.-L., Zervakis, G. I., & Karnaouri, A. (2026). Harnessing the potential of basidiomycetes for sustainable degradation of plastics and catalytic upcycling. *Biotechnology for the Environment*, 3(1), 3. <https://doi.org/10.1186/s44314-026-00038-9>
 17. Hao, M.-J., Wu, D., Xu, Y., Tao, X.-M., Li, N., & Yu, X.-W. (2022). A novel endo-polygalacturonase from *Penicillium rolfsii* with prebiotics production potential: cloning, characterization and application. *Foods*, 11(21), 3469. <https://doi.org/10.3390/foods11213469>
 18. Heidarizadeh, M., Fathi Rezaei, P., & Shahabivand, S. (2018). Novel pectinase from *Piriformospora indica*, optimization of growth parameters and enzyme production in submerged culture condition. *Turkish Journal of Biochemistry*, 43(3), 289–295. <https://doi.org/10.1515/tjb-2017-0192>
 19. Ibrahim, D., Salikin, N.-H., Lim, S. H., Ahmad, R., & Welosamy, H. (2013). Pomelo peels as alternative substrate for extracellular pectinase production by *Aspergillus niger* HFM-8. *Malaysian Journal of Microbiology*. <https://doi.org/10.21161/mjm.52713>
 20. Jahan, N., Shahid, F., Aman, A., Mujahid, T. Y., & Qader, S. A. U. (2017). Utilization of agro waste pectin for the production of industrially important polygalacturonase. *Heliyon*, 3(6), e00330. <https://doi.org/10.1016/j.heliyon.2017.e00330>
 21. Javaid Asad M, N. M. (2015). Production, purification and characterization of endopolygalacturonase by *Bacillus subtilis*. *Biochemistry & Analytical Biochemistry*, 04(03). <https://doi.org/10.4172/2161-1009.1000181>
 22. Jayani, R. S., Shukla, S. K., & Gupta, R. (2010). Screening of bacterial strains for polygalacturonase activity: its production by *Bacillus sphaericus* (MTCC 7542). *Enzyme Research*, 2010, 1–5. <https://doi.org/10.4061/2010/306785>
 23. Larios, G., Garcia, J. M., & Huitron, C. (1989). Endopolygalacturonase production from untreated lemon peel by *Aspergillus* sp. CH-Y-1043. *Biotechnology Letters*, 11(10), 729–734. <https://doi.org/10.1007/BF01044106>
 24. Liu, J., Liu, W., Cai, Y., Liao, X., Huang, Q., & Liang, X. (2014). Laccase production by *Trametes hirsuta*, characterization, and its capability of decoloring chlorophyll. *Polish Journal of Microbiology*, 63(3), 323–333.
 25. Martins, E. da S., Leite, R. S. R., da Silva, R., & Gomes, E. (2012). Production and characterization of polygalacturonase from thermophilic *Thermoascus aurantiacus* on submerged fermentation. *Annals of Microbiology*, 62(3), 1199–1205. <https://doi.org/10.1007/s13213-011-0360-0>
 26. Mathew, A., Eldo, A. N., & Molly, A. G. (2008). Optimization of culture conditions for the production of thermostable polygalacturonase by *Penicillium* SPC-F 20. *Journal of Industrial Microbiology & Biotechnology*, 35(9), 1001–1005. <https://doi.org/10.1007/s10295-008-0375-0>
 27. Oumer, O. J., & Abate, D. (2018). Comparative studies of pectinase production by *Bacillus subtilis* strain Btk 27 in submerged and solid-state fermentations. *BioMed Research International*, 2018, 1–10. <https://doi.org/10.1155/2018/1514795>
 28. Patel, D. G. B., Joshi, D. A., & Shah, D. K. R. (2024). A review on pectinolytic enzyme. In *Futuristic Trends in Biotechnology Volume 3 Book 4* (pp. 244–256). Iterative International Publishers, Selfypage Developers Pvt Ltd. <https://doi.org/10.58532/V3BJBT4P2CH5>
 29. Patidar, M. K., Nighojkar, S., Kumar, A., & Nighojkar, A. (2018). Pectinolytic enzymes-solid state fermentation, assay methods and applications in fruit juice industries: a review. *3 Biotech*, 8(4), 199. <https://doi.org/10.1007/s13205-018-1220-4>
 30. Ravichandran, A., Kolte, A., Dhali, A., Gopinath, S., & Srid, M. (2022). *Transcriptomic analysis of the white-rot basidiomycete Lentinus squarrosulus to provide insights into its lignocellulose biodegradation ability*. <https://doi.org/10.21203/rs.3.rs-1136812/v1>
 31. Rozendo, A. S., Vandenberghe, L. P. de S., de Mattos, P. B. G., Rogez, H. L. G., & Soccol, C. R. (2024). Pectinase production from cocoa pod husk in submerged fermentation and its application in the clarification of apple juice. *Fermentation*, 10(7), 337. <https://doi.org/10.3390/fermentation10070337>
 32. Samreen, P., Mangipudi, M., Grover, S., Rajan, H., & G, S. (2019). Production of pectinases and pectinolytic enzymes: microorganisms, cultural conditions and substrates. *Advances in Biotechnology &*

- Microbiology*, 14(2).
<https://doi.org/10.19080/AIBM.2019.14.555884>
33. Serrat, M., Bermúdez, R. C., & Villa, T. G. (2004). Considerations on endopolygalacturonase activity and determination of comparison ratios with emphasis on the influence of the degree of substrate esterification. *Journal of Agricultural and Food Chemistry*, 52(6), 1534–1538. <https://doi.org/10.1021/jf030415e>
34. Sharma, N., Patel, S. N., Rai, A. K., & Singh, S. P. (2024). Biochemical characterization of a novel acid-active endopolygalacturonase for pectin depolymerization, pectic-oligomer production, and fruit juice clarification. *International Journal of Biological Macromolecules*, 267, 131565. <https://doi.org/10.1016/j.ijbiomac.2024.131565>
35. Shrestha, S., Chio, C., Khatiwada, J. R., Mokale Kognou, A. L., Chen, X., & Qin, W. (2023). Optimization of cultural conditions for pectinase production by *Streptomyces* sp. and characterization of partially purified enzymes. *Microbial Physiology*, 33(1), 12–26. <https://doi.org/10.1159/000528257>
36. Singh nee' Nigam, P., & Pandey, A. (Eds.). (2009). Biotechnology for agro-industrial residues utilisation. In *Biotechnology for Agro-Industrial Residues Utilisation* (pp. 197–226). Dordrecht: Springer Netherlands. <https://doi.org/10.1007/978-1-4020-9942-7>
37. Vasina, D. V., Pavlov, A. R., & Koroleva, O. V. (2016). Extracellular proteins of *Trametes hirsuta* st. 072 induced by copper ions and a lignocellulose substrate. *BMC Microbiology*, 16(1), 106. <https://doi.org/10.1186/s12866-016-0729-0>
38. Zhou, X.-W., Su, K.-Q., & Zhang, Y.-M. (2012). Applied modern biotechnology for cultivation of *Ganoderma* and development of their products. *Applied Microbiology and Biotechnology*, 93(3), 941–963. <https://doi.org/10.1007/s00253-011-3780-7>
39. Zubyk, P., Klechak, I., Dzyhun, L., Titova, L., & Linovytska, V. (2025). Utilization of lignocellulosic waste from the agro-food industry by edible basidiomycetes *Pleurotus* spp. *Journal of Microbiology, Biotechnology and Food Sciences*, e11647. <https://doi.org/10.55251/jmbfs.11647>
40. Zubyk, P. R., & Klechak, I. R. (2024). Evaluation of pectinolytic activity and growth of *Trametes versicolor* and *Trametes ochracea* strains on pectin-containing agarified medium. *Biotechnologia Acta*, 17(5), 33–44. <https://doi.org/10.15407/biotech17.05.033>

СКРИНІНГ УМОВ КУЛЬТИВУВАННЯ *TRAMETES HIRSUTA* ІВК 1569 ДЛЯ ОТРИМАННЯ ЕНДОПОЛІГАЛАКТУРОНАЗИ

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У роботі досліджено умови синтезу ендopolігалактуронази штамом *Trametes hirsuta* 1569 при глибинному культивуванні. Встановлено, що максимальна активність ферменту досягалася на 9 добу, після чого спостерігалось її зниження. Серед досліджених джерел вуглецю найбільш ефективним виявився буряковий жом, тоді як пивна дробина не індукувала синтез ферменту. Оптимальна концентрація бурякового жому становила 10 г/дм³, що забезпечувало високий рівень активності без подальшого ефекту насичення. Максимальний синтез ферменту спостерігався при використанні сечовини та сульфату амонію як джерел азоту, причому оптимальна концентрація (NH₄)₂SO₄ становила 1,0 г/дм³. Найсприятливіше для синтезу ендopolігалактуронази значення рН середовища знаходилось у межах 5–7 з максимумом за рН 6. Помірна швидкість перемішування (120 об/хв) та внесення 5 % посівного матеріалу забезпечують максимальний синтез ферменту. Отримані результати свідчать про доцільність використання лігноцелюлозних відходів, зокрема бурякового жому, для біосинтезу ендopolігалактуронази та можуть бути використані для оптимізації біотехнологічних процесів.

Ключові слова: біотехнологія, пектинолітичні ферменти, параметри культивування, склад поживного середовища, вихід ферменту.

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