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Screening of *Trametes hirsuta* IBK 1569 culture conditions for the production of pectin lyase as a component of the pectinolytic complex

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Abstract

Pectin lyases are essential enzymes of the pectinolytic complex that catalyze the degradation of pectic substances and possess significant biotechnological potential. This study investigated conditions for pectin lyase biosynthesis by *Trametes hirsuta* IBK 1569 under submerged cultivation. The effects of cultivation time, lignocellulosic waste as carbon sources, their concentration and particle size, nitrogen source and concentration, initial pH, agitation speed, and inoculum size on pectin lyase activity in the culture broth of *T. hirsuta* IBK 1569 were investigated. Although the maximum activity of pectin lyase was achieved on the 5th day of cultivation, from the point of view of developing a complex enzyme preparation, it was advisable to cultivate the macromycete for 9 days. Sugar beet pulp and pomelo peels were the most effective carbon sources; apple pomace provided a statistically similar level of activity. The optimal concentration of sugar beet pulp was 10 g/dm^3. The highest activity was recorded using a particle size of 0–1 mm, though no significant differences were observed within the 0–3 mm range. Ammonium salts were the most effective nitrogen sources, with the optimal ammonium sulfate concentration being 1.0 g/dm^3. An initial pH of 6.0 and 120 rpm were selected for further studies considering the complex production of pectinolytic enzymes. The optimal inoculum size was 5%. Sugar beet pulp is a promising carbon source and inducer for pectin lyase synthesis by *T. hirsuta* IBK 1569. The obtained results can be applied to optimize the biotechnological production of pectinolytic enzyme preparations.

Keywords: lyase, submerged cultivation, enzyme activity, lignocellulosic waste, cultivation parameters, biotechnology.

Abbreviations: PL, pectin lyase; PLA pectin lyase activity; relPLA, relative pectin lyase activity.

1. Introduction

Pectin lyase (EC 4.2.2.10) belongs to the enzymes of the pectinolytic complex and catalyzes the degradation of highly esterified pectin by β-elimination of α-1,4-glycosidic bonds between D-galacturonic acid residues. Unlike polygalacturonases, which perform hydrolytic degradation of pectic substances, pectin lyases cleave pectin without prior demethoxylation, forming unsaturated oligogalacturonides (Yadav et al., 2009; Zheng et al., 2021).

The ability of pectin lyases to act directly on highly methoxylated pectin determines their important role in degrading plant cell wall pectic components. Consequently, these enzymes have significant practical application in the food industry, primarily in fruit juice clarification, reducing the viscosity of pectin-containing media, improving filtration, and increasing juice yield (Poturcu et al., 2017). Besides the food industry, pectin lyases are used in the textile processing of plant fibers, maceration of plant materials, biological treatment of fabrics, and agro-industrial waste processing, due to their ability to selectively degrade pectic cell wall components without using harsh chemical reagents (Zheng et al., 2021).

Microorganisms remain the main producers of pectin lyases, predominantly bacteria of the genera *Bacillus* (Ouattara et al., 2008) and *Pseudomonas* (Gül et al., 2025). Additionally, the biosynthesis of these enzymes by micromycetes has been reported. For instance, *Aspergillus brasiliensis* has been shown to synthesize pectin lyase under submerged cultivation in media containing agro-industrial waste (Pili et al., 2018).

The synthesis level of fungal pectinases significantly depends on the nutrient medium composition, carbon and nitrogen sources, initial pH, cultivation time, and aeration conditions (El Enshasy et al., 2018). Using pectin-containing agro-industrial waste is a promising approach to reduce the cost of biotechnological pectinolytic enzyme production. Such substrates simultaneously serve as a carbon source and a natural inducer of pectinase synthesis (Heerd et al., 2014). Citrus waste is also widely considered a promising pectin-containing raw material for biotechnological processes, particularly due to the high pectin content in fruit peels (Ravindran et al., 2018). However, the regional characteristics of Ukraine do not allow them to be considered an available carbon source.

Compared to micromycetes, basidiomycetous macromycetes are significantly less studied as pectin lyase producers. At the same time, they possess a potent complex of extracellular enzymes capable of degrading plant cell wall polymers. Genomic studies of wood-decaying fungi confirm the presence of a wide range of enzymes associated with plant biomass degradation in basidiomycetes (Floudas et al., 2012; Zubyk et al., 2026a). Only a limited number of studies are dedicated to pectic substance-degrading lyases produced specifically by macromycetes. Available studies focus on the cultivation of *Fomitopsis cytisina* (Miyairi et al., 2001), *Hypsizygus ulmarius* (Jatav et al., 2014), *Pleurotus ostreatus* (Rashad et al., 2009), and *Schizophyllum commune* (Mehmood et al., 2019).

Of particular interest are representatives of the genus *Trametes*, known as active producers of lignolytic, cellulolytic, and hemicellulolytic enzymes. Literature data confirm the ability of this genus to form an active secretome involved in plant biomass bioconversion (Machado et al., 2020). The synthesis of a broad spectrum of extracellular proteins during cultivation on a lignocellulosic substrate has been demonstrated for *Trametes hirsuta* (Vasina et al., 2016). Our previously obtained results on *T. hirsuta* IBK 1569 indicate the potential of this strain for studying its pectinase activity (Zubyk et al., 2026b). Thus, investigating *T. hirsuta* IBK 1569 as a pectin lyase producer is highly relevant.

The aim of this work was to investigate the effect of main cultivation parameters on pectin lyase synthesis by the fungus *Trametes hirsuta* IBK 1569 in submerged culture suitable for further development of a complex pectinolytic enzyme preparation.

2. Materials and methods

Object of study. The strain *Trametes hirsuta* IBK 1569, obtained from the Mushroom Culture Collection of the M.G. Kholodny Institute of Botany, NAS of Ukraine (IBK) (Bisko et al., 2024), was used in this work. The culture was maintained on agarized barley malt extract (8° Balling) at 4 °C.

Inoculum preparation. The inoculum was grown in 250 cm^3 flasks containing 50 cm^3 of glucose-peptone-yeast medium according to (Bondaruk et al., 2025) at (28 ± 2) °C for 7 days.

Basic cultivation conditions. The basic nutrient medium consisted of (g/dm^3): pectin (10.0), NH_4NO_3 (1.0), KH_2PO_4 (1.0), $MgSO_4 \cdot 7H_2O$ (0.5), $FeSO_4 \cdot 7H_2O$ (0.005), $ZnSO_4 \cdot 7H_2O$ (0.0044), $CaCl_2$ (0.0055), with an initial pH of 6.5. The medium was autoclaved at 121 °C for 15 min (Danylyak et al., 1989). Submerged cultivation was carried out in 250 cm^3 Erlenmeyer flasks containing 50 cm^3 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 pectin lyase yield (Oumer & Abate, 2017). At each stage, only one parameter was changed, while other factors were maintained at the levels determined as optimal in the previous stages of this experiment. At some stages, the final selected value was determined not only by the maximum activity of pectin lyase, but also by other enzymes of the pectinolytic complex for further development of a complex pectinolytic enzyme preparation. The concentration of salts KH_2PO_4, $MgSO_4 \cdot 7H_2O$, $FeSO_4 \cdot 7H_2O$, $ZnSO_4 \cdot 7H_2O$, $CaCl_2$ and the cultivation temperature were set at the initial level. The parameters were varied in the following order: cultivation time, carbon source, its concentration and size, nitrogen source, its concentration, initial pH, agitation speed, and inoculum size.

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

Carbon source, its size, and concentration. Agricultural and industrial wastes were evaluated as alternative carbon sources at a concentration of 1% (w/v, dry weight): sugar beet pulp, grape pomace, pomegranate peels, brewer's spent grain (barley), brewer's spent grain (wheat), corn husk, mandarin peels, pomelo peels, and apple pomace. The waste was dried at 60 °C and crushed to a size of 0–1 mm. The determined optimal carbon source was used for further studies. The concentration of the selected carbon source was determined by cultivating the basidiomycetous macromycete on a nutrient medium with varying contents of this component: from 5 g/dm^3 to 35 g/dm^3 with a 5 g/dm^3 step. The optimal concentration of the carbon source was used in subsequent studies. The particle size of the selected carbon source was chosen 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 subsequent studies.

Nitrogen source and concentration. The qualitative composition of the nitrogen source was tested at a concentration of 1% (w/v). The following substances were used in this study: KNO_3, $(NH_4)_2SO_4$, NH_4NO_3, $Ca(NO_3)_2$, casein, urea, yeast extract, and peptone. The concentration of the selected nitrogen source was determined by cultivating the basidiomycetous macromycete on a nutrient medium with varying contents of this component: from 0.5 g/dm^3 to 3.0 g/dm^3 with a 0.5 g/dm^3 step. The optimal concentration of the nitrogen source was used in subsequent studies.

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

Agitation speed. The agitation speed was varied from 100 to 200 rpm with a 20 rpm step on an orbital shaker. The determined optimal agitation speed was used in subsequent studies.

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

Determination of enzyme activity. A 1.5% pectin solution was used to determine pectin lyase activity in the culture broth. The analysis was performed by a modified method presented in (Çelik et al., 2014). Into a centrifuge tube, 0.5 cm^3 of culture broth and 0.5 cm^3 of 1.5% apple pectin solution (in 0.1 M phosphate buffer, pH 7.0) are added and incubated at 30 °C for 10 min. If necessary, the culture broth can be diluted to the required extent for optimal spectrophotometer performance. 0.1 cm^3 of 1 M NaOH solution is added, kept in a water bath at 80 °C for 5 min, and cooled to room temperature. 1.2 cm^3 of 1 M HCl solution is added to the mixture, mixed, and 1 cm^3 of 0.04 M thiobarbituric acid solution is added. Kept in a water bath at 80 °C for 5 min and cooled. The cooled reaction mixture is centrifuged at 3000 rpm for 10–15 min. Culture broth, previously kept at 105 ± 5 °C for 10–15 min to inactivate the enzyme, is used as a control solution. Optical density is measured at 550 nm against the control solution.

The pectin lyase activity (PLA, U/cm^3) of pectin lyase is calculated by the formula:

$$PLA = \frac{A \cdot f \cdot V_t \cdot 10^6}{\varepsilon \cdot l \cdot \tau \cdot V_e} = \frac{A \cdot f \cdot 3,3 \cdot 10^6}{54000 \cdot 0,5 \cdot 10 \cdot 0,5} = 24,44 \cdot A \cdot f$$

where A – optical density;

f – dilution factor;

V_t – total volume of the reaction mixture (3,3 cm^3);

– molar extinction coefficient of reaction products at 550 nm (54000 $M^{-1} \cdot cm^{-1}$) (Nedjma et al., 2001);

l – cuvette thickness (0,5 cm);

τ – incubation time (10 min);

V_e – volume of enzyme solution or culture fluid (0,5 cm^3).

One unit of PL activity (EU) was defined as the amount of enzyme which produces 1 μmol of unsaturated galacturonide per minute (Çelik et al., 2014).

After that, the relative pectin lyase activity was calculated according to the formula (Oumer & Abate, 2018):

$$relPLA = \frac{PLA}{\max(PLA)} \times 100\%$$

Statistical analysis. Statistical data analysis was performed using Duncan's multiple range test. All experiments were performed in triplicate; results are presented as M ± m (mean ± standard deviation), obtained from three measurements (n = 3). Results were considered statistically significantly different at a p-value < 0.05. Letters a, b, c, d... indicate differences between samples: $p < 0.05$ according to Duncan's test.

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

3. Results

The dynamics of pectin lyase synthesis by the *Trametes hirsuta* IBK 1569 strain were investigated over 504 h of cultivation in the basic nutrient medium (Fig. 1).

Enzyme activity reached its maximum on the 5th day of cultivation (2.6 ± 0.1 U/cm^3). Subsequent cultivation for 4 days was accompanied by a slight decrease in PL activity values. Starting from the 11th day, a sharp decline in activity was observed, reaching complete absence by the 15th day. Thus, the 5th day of cultivation should be considered the PL-specific optimum under the initial cultivation conditions. However, for subsequent experiments, a 9-day cultivation period was used as a compromise condition, taking into account previously obtained data on other endolopygalacturonase (P. Zubyk & Klechak, 2026). Therefore, the following stages should be interpreted as screening of cultivation parameters under conditions relevant to the development of a complex pectinolytic preparation rather than as strict optimization of pectin lyase activity alone.

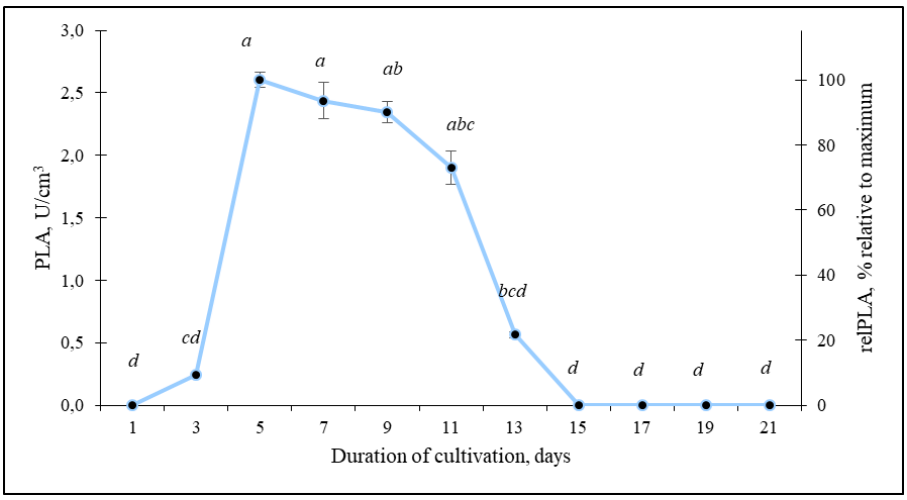

Fig. 1. Dynamics of pectin lyase synthesis by *Trametes hirsuta* 1569. Data are presented as mean ± standard deviation, n = 3. Letters a, b, c, d indicate differences between samples: $p < 0.05$ according to Duncan's test.

The effect of lignocellulosic waste as carbon sources, their concentration, and size on PL synthesis by *T. hirsuta* IBK 1569 is shown in Fig. 2.

The highest PL activity was recorded using sugar beet pulp and pomelo peels (2.2–3.4 U/cm³). Statistically similar values ($p > 0.05$) to the maximum were observed in the medium with apple pomace. Meanwhile, PL activity when using mandarin peels, corn husks, and grape pomace was 60–80% lower. The other carbon sources investigated in this study did not induce pectin lyase synthesis. Therefore, sugar beet pulp was selected as the carbon source for further experiments.

When investigating the concentration effect, a clear peak was observed in the range of 10–15 g/dm³ of sugar beet pulp. Increasing the substrate concentration above 20 g/dm³ led to a significant decrease in enzyme activity. Thus, a carbon source concentration of 10 g/dm³ was chosen as optimal for further studies.

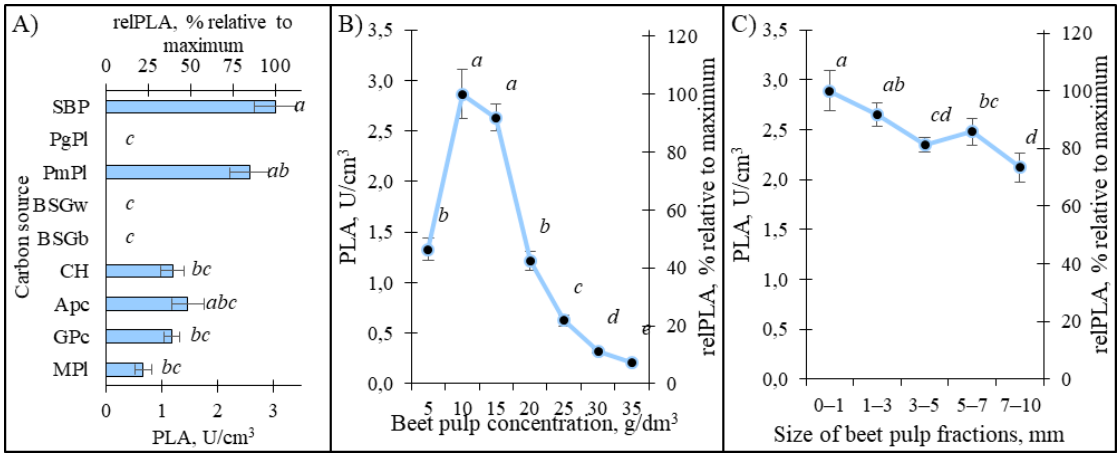

Fig. 2. Effect of carbon source type (A), its concentration (B) and particle size (C) on pectin lyase synthesis by *Trametes hirsuta* 1569. Data are presented as mean ± standard deviation, n = 3. Letters a, b, c, d... indicate differences between samples: $p < 0.05$ according to Duncan's test. *Note:* SBP – sugar beet pulp; PgPl – pomegranate peels; PmPl – pomelo peels; BSGw – brewer's spent grain (wheat); BSGb – brewer's spent grain (barley); CH – corn husk; Apc – apple pomace; GPc – grape pomace; MPl – mandarin peels.

The PL activity of *T. hirsuta* IBK 1569 also depended insignificantly on the sugar beet pulp particle size (Fig. 2.C). The maximum PL activity value was recorded using particles of 0–1 mm in

size (2.9 ± 0.2 U/cm³). A lower degree of comminution negatively affected PL activity. PL activity values gradually decreased by 10–30% with an increase in the size of the sugar beet pulp fractions. It should be noted that in the particle size range of 0–3 mm, PL activity was the same ($p > 0.05$); therefore, the 1–3 mm fraction was selected for further studies.

Besides the carbon source, the nitrogen source played a significant role in PL synthesis (Fig. 3.A). The highest activity of the studied enzyme in the culture broth was observed after cultivating *T. hirsuta* IBK 1569 on a medium with ammonium salts (2.9–3.5 U/cm³). Organic sources (except urea) and potassium nitrate demonstrated values statistically similar to the maximum ones ($p > 0.05$). Ammonium sulfate was selected as the best nitrogen source for PL synthesis due to the comprehensive evaluation of the pectinolytic complex enzymes.

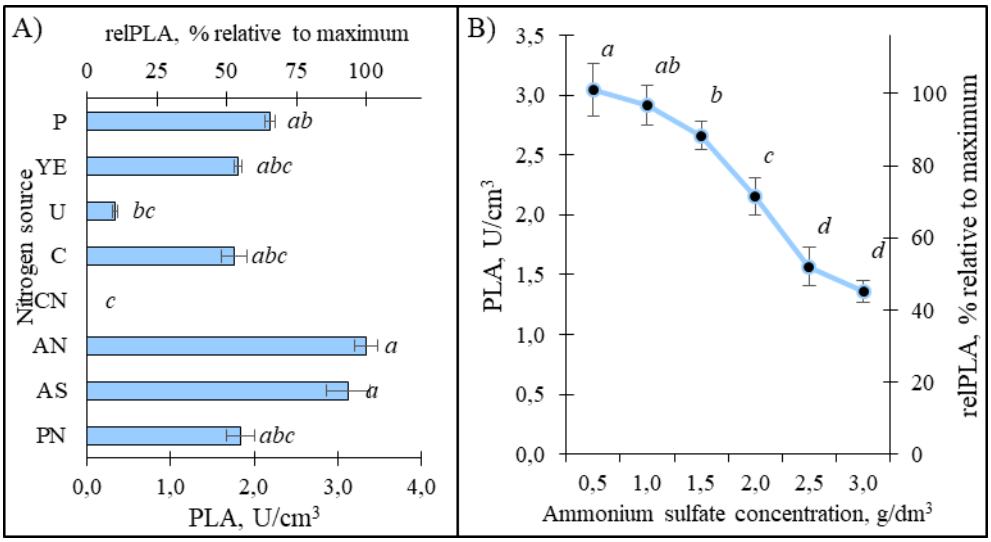

Fig. 3. Effect of nitrogen source type (A) and its concentration (B) on pectin lyase synthesis by *Trametes hirsuta* 1569. Data are presented as mean ± standard deviation, n = 3. Letters a, b, c, d indicate differences between samples: $p < 0.05$ according to Duncan's test. *Note:* PN – potassium nitrate; AS – ammonium sulfate; AN – ammonium nitrate; CN – calcium nitrate; K – casein; U – urea; YE – yeast extract; P – peptone.

Next, the optimal ammonium sulfate concentration in the nutrient medium was determined (Fig. 3.B). Maximum PL activity was recorded at low concentrations: 0.5 g/dm³ and 1.0 g/dm³ – 3.0 ± 0.2 U/cm³ and 2.9 ± 0.2 U/cm³, respectively, which exceeded the values at other concentrations by 25–50% ($p < 0.05$). However, in our previous studies of exopolygalacturonase, 1.0 g/dm³ of ammonium sulfate provided the highest activity level; therefore, this value was chosen as the optimal nitrogen source concentration.

A significant effect of the nutrient medium pH on PL synthesis by *T. hirsuta* IBK 1569 was noted (Fig. 4.A). The highest PL activity values (3.2–4.0 U/cm³) were observed in the alkaline range. Therefore, this range can be considered optimal for pectin lyase accumulation under the tested conditions. A more acidic medium reduced PL activity by 20–95%. Nevertheless, an initial pH of 6.0 was selected for further experiments as a compromise value, taking into account the production of other enzymes (e.g. endopolygalacturonase) of the pectinolytic complex (P. Zubyk & Klechak, 2026).

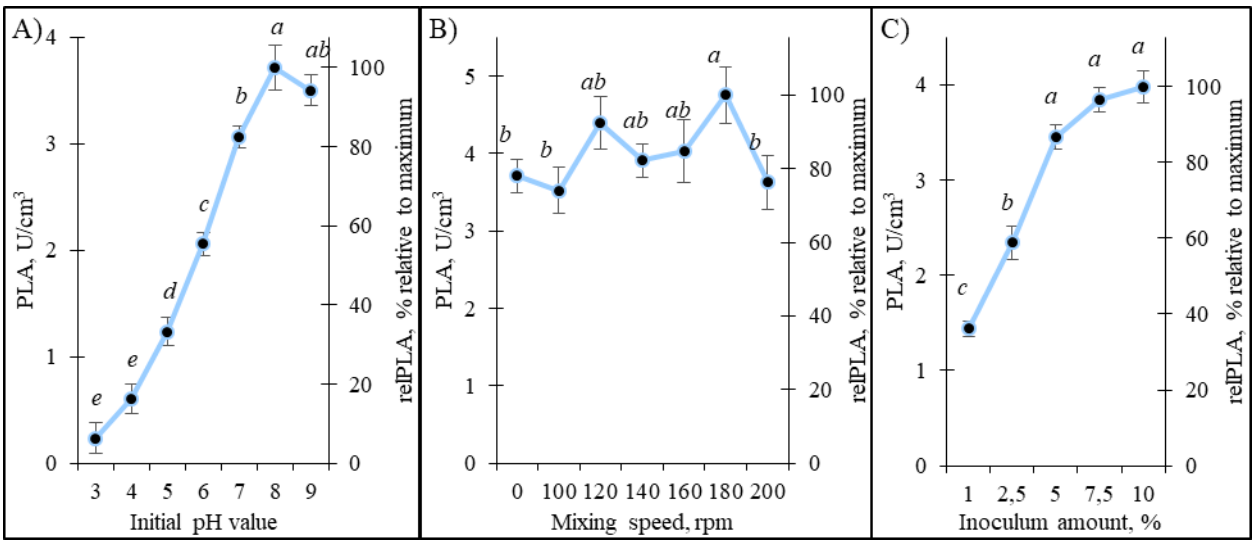

Fig. 4. Effects of initial pH (A), agitation rate (B) and inoculum size (C) on pectin lyase synthesis by *Trametes hirsuta* 1569. Data are presented as mean ± standard deviation, n = 3. Letters a, b, c, d indicate differences between samples: $p < 0.05$ according to Duncan's test.

The effect of agitation to intensify PL yield was also investigated. According to Fig. 4.B, the maximum enzymatic activity value (4.8 ± 0.4 U/cm^3) was observed with active agitation at 180 rpm. However, because PL activity remained statistically comparable within the 120–180 rpm range ($p > 0.05$) and considering the broader goal of obtaining a complex pectinolytic enzyme preparation, 120 rpm was selected for further studies. Thus, further studies were conducted at an agitation speed of 120 rpm.

A significant effect of the inoculum size on enzyme synthesis was also noted (Fig. 4.C). The highest PL activity was recorded in the range of 5–10% inoculum. Further reductions were accompanied by a 2–3-fold decrease in enzymatic activity. The obtained results indicate that the optimal inoculum size for further studies of the macromycete *T. hirsuta* IBK 1569 is 5%.

4. Discussion

The established dynamics of pectin lyase synthesis by *Trametes hirsuta* IBK 1569 indicate early enzyme accumulation, peaking on the 5th day of cultivation, followed by a gradual decrease in activity. This pattern is typical for induced extracellular fungal enzymes, whose synthesis is activated in response to a polymeric substrate. The subsequent decline may be associated with inducer depletion, medium pH changes, or proteolytic inactivation (El Enshasy et al., 2018; Vasina et al., 2016). *T. hirsuta* has previously been shown to secrete a broad spectrum of extracellular proteins when grown on a lignocellulosic substrate, aligning with the obtained results regarding PL production by this strain (Vasina et al., 2016).

The highest PL activity observed using sugar beet pulp, pomelo peels, and apple pomace confirms the crucial role of pectin-containing substrates in inducing enzyme synthesis. Pectin lyases cleave methoxylated pectin via β-elimination; therefore, the presence of pectin or pectin-rich plant materials in the medium is a logical factor stimulating their biosynthesis (Zeuner et al., 2020; Zheng et al., 2021). The effectiveness of citrus peels and apple pomace as inducers is consistent with literature data on using agro-industrial waste for pectin lyase synthesis. Specifically, PL production in submerged culture on media with agro-industrial residues, including orange peel, has been demonstrated for *Aspergillus brasiliensis* (Pili et al., 2018). Pectin lyase production using agricultural waste has also been described for *Penicillium expansum* (Atalla et al., 2019).

Sugar beet pulp is also a suitable substrate for such processes as it contains cellulose, hemicelluloses, and pectic substances, making it a promising raw material for producing the

corresponding hydrolytic enzymes (Heerd et al., 2014; Marzo-Gago et al., 2023). This confirms the feasibility of using pectin-containing agricultural and food industry wastes as low-cost inducers and carbon sources for PL biosynthesis.

The optimal sugar beet pulp concentration in the range of 10–15 g/dm^3 indicates substrate-dependent PL synthesis induction. Increasing the pectin-containing substrate concentration to a certain level enhances inducer availability; however, an excess of solid plant material can limit mass transfer, increase medium viscosity, and impair aeration. Similar patterns have been described during the optimization of fungal pectinase synthesis in submerged cultivation, where an excessive increase in pectin or substrate concentration did not result in further enzymatic activity growth (El Enshasy et al., 2018; Marzo-Gago et al., 2023). Particle size can have a comparable effect. Higher activity when using finer fractions can be attributed to the substrate's increased specific surface area, better contact of the mycelium with the pectin-containing raw material, and more efficient induction of the enzyme system (Liadi et al., 2026).

The nitrogen source significantly influenced PL synthesis. The highest activity values obtained using ammonium salts and similar figures for organic nitrogen sources indicate the flexibility of the strain's nitrogen metabolism. For fungal pectinases, it has been shown that the type of nitrogen source can substantially alter the enzyme production level, and either organic or inorganic nitrogen can be optimal depending on the producer species and strain (Marzo-Gago et al., 2023). Selecting ammonium sulfate is technologically justified, as it provided a high level of PL activity while serving as a simple and accessible nutrient medium component.

Maximum PL activity at ammonium sulfate concentrations of 0.5–1.0 g/dm^3 suggests that a low mineral nitrogen level is favorable for enzyme synthesis. Decreased activity at higher concentrations may result from a metabolic shift toward biomass growth or the repression of target enzyme synthesis. A similar relationship between nitrogen source concentration and pectinase production was shown for *Aspergillus niger* LFP-1, where nitrogen nutrition optimization significantly affected the enzyme yield (Marzo-Gago et al., 2023).

PL synthesis by *T. hirsuta* IBK 1569 substantially depended on the initial pH of the medium and was highest in the alkaline range. This pattern is biochemically justified, as pectin lyases belong to a group of enzymes that perform β-eliminative pectin cleavage, and many enzymes in this group exhibit higher activity in neutral or alkaline media (Zheng et al., 2021). An activity optimum in the neutral-alkaline zone has also been described for certain fungal pectin lyases, particularly for the *Aspergillus giganteus* pectin lyase, which has an optimum pH of 8.5 (Pedrolli & Carmona, 2009).

The positive effect of active agitation on PL synthesis distinguishes this enzyme from some other components of the *T. hirsuta* IBK 1569 pectinolytic complex. The maximum activity at 180 rpm may be related to improved oxygen mass transfer, more uniform substrate particle distribution, and more intensive mycelium-inducer contact. Similarly, for *A. niger* LFP-1, increasing the agitation speed enhanced pectinase production (Marzo-Gago et al., 2023). Nevertheless, selecting 120 rpm for further studies is appropriate from the perspective of complex pectinolytic enzyme production, as PL activity remained statistically similar within the 120–180 rpm range.

The inoculum size was a critical factor in PL synthesis. The highest activity values at 5–10% inoculum indicate that rapid formation of active mycelial biomass and colonization of the pectin-containing substrate are necessary for efficient enzyme production. A decrease in inoculum size was accompanied by a 2–3-fold reduction in PL activity, which may be caused by a prolonged lag phase, slower substrate assimilation, and delayed enzyme system induction. The importance of optimizing the inoculum size to maximize pectinase yield is well documented for *A. niger*, where the optimal initial inoculum volume ensured the highest enzymatic activity (Marzo-Gago et al., 2023).

It should be noted that in this study, volumetric PL activity was used as the main selection criterion because the work was focused on screening cultivation conditions for extracellular enzyme accumulation in the culture broth. Therefore, the selected parameters should not be interpreted as

final conditions providing maximum specific activity or biomass-normalized productivity, but rather as conditions favorable for obtaining a crude pectinolytic enzyme preparation.

5. Conclusions

The study demonstrated that *Trametes hirsuta* IBK 1569 is capable of producing pectin lyase under submerged cultivation. The accumulation of pectin lyase was strongly dependent on cultivation time and reached its maximum on the 5th day of cultivation, after which a gradual decrease in enzymatic activity was observed. Therefore, the 5th day can be considered the most favorable cultivation time for pectin lyase accumulation under the initial experimental conditions.

Among the investigated lignocellulosic wastes, sugar beet pulp, pomelo peels, and apple pomace provided the highest pectin lyase activity. Sugar beet pulp was selected for further experiments due to its high efficiency, availability, and practical relevance as an agro-industrial residue. The most favorable concentration of sugar beet pulp was 10 g/dm^3, whereas further increases in substrate concentration resulted in reduced pectin lyase activity. The highest activity was observed when using the 0–1 mm particle fraction; however, no statistically significant differences were found within the 0–3 mm range, indicating that moderate grinding of the substrate is sufficient for further technological application.

The nitrogen source and its concentration also influenced pectin lyase accumulation. Ammonium salts provided the highest activity values, while several organic nitrogen sources and potassium nitrate showed statistically comparable results. Ammonium sulfate at a concentration of 1.0 g/dm^3 was selected as a suitable nitrogen source for further cultivation, taking into account both pectin lyase activity and the broader production of enzymes of the pectinolytic complex.

The highest pectin lyase activity was recorded in the alkaline pH range and at an agitation speed of 180 rpm. These conditions may therefore be considered the most favorable specifically for pectin lyase accumulation under the tested conditions. However, an initial pH of 6.0 and an agitation speed of 120 rpm were selected for subsequent studies as compromise parameters suitable for the further development of a complex pectinolytic enzyme preparation. The optimal inoculum size was 5%, as lower inoculum levels resulted in a marked decrease in enzyme activity.

Thus, the obtained results should be interpreted as a screening-based selection of cultivation parameters rather than as final optimization of pectin lyase production alone. The selected conditions – sugar beet pulp at 10 g/dm^3, ammonium sulfate at 1.0 g/dm^3, initial pH 6.0, agitation speed of 120 rpm, and inoculum size of 5% – provide a basis for further optimization of complex pectinolytic enzyme production by *T. hirsuta* IBK 1569. Further studies should include normalization of enzyme activity to fungal biomass and extracellular protein content to clarify whether the observed changes reflect specific induction of pectin lyase synthesis or differences in overall fungal growth and secretion intensity.

The obtained results can be applied to further optimize the production technology of complex pectinolytic enzyme preparations. Since enzyme activity was evaluated mainly as volumetric activity in the culture broth, further studies may include normalization to biomass and extracellular protein content for a more detailed biochemical characterization of PL biosynthesis by *T. hirsuta* IBK 1569.

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Author contributions.

Planning: PZ, IK. Conducting experiments, data analysis, writing the original draft, figures, and referencing: PZ. Writing-reviewing, editing: ON, IK. Translation: ON. Management: IK. All authors participated in revising the manuscript and approved the submitted version.

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Data availability. All data are available on request.

Declarations

Conflict of interest. The authors have no competing interests to declare relevant to this article's content.

Research involving human participants and/or animals. Not applicable.

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Анотація

Пектинліази є важливими ферментами пектинолітичного комплексу, що каталізують розщеплення пектинових речовин і мають значний біотехнологічний потенціал. Метою роботи було дослідити умови біосинтезу пектинліази штамом *Trametes hirsuta* ІВК 1569 за умов глибинного культивування. Досліджували вплив тривалості культивування, лігноцелюлозних відходів як джерел вуглецю, їх концентрації та розміру, джерела і концентрації азоту, вихідного значення pH, швидкості перемішування та кількості посівного матеріалу на пектинліазну активність у культуральній рідини *T. hirsuta* ІВК 1569. Активність визначали спектрофотометрично з використанням тіобарбітурової кислоти. Хоча максимальна активність пектинліази досягалася на 5 добу культивування, з точки зору розробки комплексного ферментного препарату доцільним було культивувати макроміцет упродовж 9 діб. Найефективнішими джерелами вуглецю були буряковий жом і шкірки помело; яблучні вичавки забезпечували статистично подібний рівень активності. Оптимальна концентрація бурякового жому становила 10 г/дм^3. Найвищу активність зафіксовано при використанні частинок розміром 0–1 мм, проте в діапазоні 0–3 мм достовірних відмінностей не встановлено. Найефективнішими джерелами азоту були солі амонію, а оптимальна концентрація амоній сульфату становила 1.0 г/дм^3. Для подальших досліджень обрано pH 6.0 і 120 об/хв з урахуванням комплексної продукції пектинолітичних ферментів. Оптимальна кількість посівного матеріалу становила 5 %. Буряковий жом є перспективним джерелом вуглецю та індуктором синтезу пектинліази *T. hirsuta* ІВК 1569. Отримані результати можуть бути використані для оптимізації біотехнологічного одержання пектинолітичних ферментних препаратів.

Ключові слова: ліаза, глибинне культивування, активність ферменту, лігноцелюлозні відходи, параметри культивування, біотехнологія.