



Research Article

Valorization of Fruit Peel Waste for Pectinase Enzyme Production Using Soil-Dwelling Filamentous Fungi

Satwika Das¹, Bhavik Jain¹, K Prashanth Kumar¹, Pardeep Kaur², Ashish A Prabhu^{1*}

¹Bioprocess Development Laboratory, Department of Biotechnology, National Institute of Technology Warangal, Warangal, Telangana, India 506004

²Department of Biotechnology, University Institute of Biotechnology, Chandigarh University, Mohali, Punjab, India 140413

*Corresponding author: Dr. Ashish A. Prabhu: ashishp@nitw.ac.in

Abstract

India's fruit processing industry suffers from significant post-harvest losses due to inadequate infrastructure and poor management. Around 30 – 35 % of fruit is wasted annually, which leads to loss of immense nutritive potential. Only 1.8 % of the horticultural produce is currently processed, however, the growing global market for processed fruit highlights an urgent need for sustainable solutions. A major by-product of the fruit processing industry is citrus waste, rich in pectin, a valuable biopolymer used across food, pharmaceutical and other industrial sectors. This study focuses on the screening and identification of potent fungal strains for pectinase production, specifically targeting the valorization of citrus peels and pomace. A robust strain of *Aspergillus fumigatus* was identified to produce pectinase activity of 4.44 U/ml while growing on orange peel. By optimizing the growth parameters for enhanced enzyme production, the pectinase activity was enhanced to 8.85 U/ml and characterization of the crude fungal pectinase further enhanced the enzyme activity. The final optimized parameters were found to be pH 7, temperature 45 °C in the presence of 60 mM MgCl₂. This research aims to enable broader industrial applications of the pectinase enzyme by creating a cost-effective and sustainable bioprocess. The study ultimately seeks to transform fruit processing waste into value-added products, contributing to both environmental sustainability and economic growth.

Keywords: Orange peel valorization, *Aspergillus fumigatus*, pectinase, Taguchi optimization, biorefinery.

Citation: Das S, Jain B, Prashanth Kumar K, Kaur P, and Prabhu AA. (2026). Valorization of Fruit Peel Waste for Pectinase Enzyme Production Using Soil-Dwelling Filamentous Fungi. *Mycological Spectrum* 02(02):134-156. <https://doi.org/10.64993/MS.2.2.5>

Received: 14 July 2026 | **Accepted:** 10 August 2026 | **Published:** 17 August 2026.

Handling Editor: Dr. S.K. Deshmukh | **Reviewers:** Prof. Navin Kango and Dr. Sneha Nayak.

Copyright: ©2026 Das S, Jain B, Prashanth Kumar K, Kaur P, and Prabhu AA. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution, or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution, or reproduction is permitted that does not comply with these terms.



1. Introduction

Microbial enzymes are widely utilized for their diverse commercial applications. Among them, pectinase broadly consists of a group of different enzymes that hydrolyze pectin (Abdollahzadeh *et al.*, 2020). Due to their functional versatility, pectinases are extensively employed in different industries, including juice and oil extraction, fermentation, animal feed, coatings, wastewater treatment, pulp and paper production, and pharmaceuticals (Oumer, 2017). Several studies have reported the presence of pectin in large quantities in fruit wastes; amongst these, citrus peels and apple pomace are the most commercially viable sources. This is attributed to not only their high pectin content but also to their easy availability as by-products of juice industries (Abdullahi *et al.*, 2026). Additionally, India has an abundant supply of raw fruit materials; however, due to inadequate raw material management practices, nearly 30–35% of fruits are lost annually (Sajish *et al.*, 2025). The conversion of fruit waste into pectinase thus provides an economically feasible and sustainable solution to help address these losses.

Microbial pectinases have witnessed a steady increase in their demand over time, representing approximately 25% of the global food and industrial enzyme market (Kaul *et al.*, 2024). Fungi, particularly belonging to the genus *Aspergillus*, are reported to be prolific producers of extracellular enzymes, including pectinases such as polygalacturonase, pectin lyase, and pectin esterase (Rashid *et al.*, 2025). A significant portion of fungal glycoside hydrolases belongs to the GH family 28, which plays a key role in the biodegradation of the pectin backbone (Martens-Uzunova & Schaap, 2009). These enzymes act by hydrolyzing the glycosidic linkages within the recalcitrant pectin structure, resulting in the release of monomeric units such as galacturonic acid (Gawkowska *et al.*, 2018).

The pectinase enzyme can be produced using fungal strains using solid-state and submerged fermentation conditions. However, the solid-state fermentation (SSF) has gained prominence over submerged fermentation due to higher enzyme yields and better control over the biosynthesis of the enzyme (Bamidele *et al.*, 2025). The selection of fermentation operation mode, optimization of substrates, and culture conditions are essential strategies for reducing the industrial costs of enzyme production. The key factors that influence pectinase production in SSF include water activity (a_w), pH, temperature, incubation time, moisture content, inoculum size, presence of inhibitors or activators, and the type of carbon and nitrogen sources (Haile & Ayele, 2022). The purification of the crude enzyme and characterization of the enzyme are also important, as optimal fermentation conditions and improved enzyme recovery can significantly lower production costs (Siddiqui *et al.*, 2012).

This study was designed to bioprospect fungal strains for the sustainable pectinase production using fruit peels and wastes. The study evaluated the suitability of orange peels and apple



pomace for the biosynthesis of pectinase by in-house fungal strains and fungal isolates for biosynthesis of pectinase under SSF conditions. The production parameters, like pH, temperature, and metal ions, were evaluated and optimized for the enhanced production of the enzyme. The key observations of this study underscore the importance of industrially valuable enzymes like pectinase in a wide range of biotechnological applications, highlighting their sustainable and cost-effective production. The scope of the study aligns with the following targets of the UN Sustainable Development Goals, including good health and well-being (SDG 3), industry, innovation, and infrastructure (SDG 9), and sustainable production and consumption (SDG 12).

2. Materials and methods

2.1 Microorganism

Four filamentous fungal strains-*Aspergillus fumigatus*, *Aspergillus terreus*, and two unidentified strains were previously isolated from soil samples in the Bioprocess Development Laboratory, NIT Warangal, India, and preserved as glycerol stocks. The strains were revived for pectinase production using potato dextrose agar (PDA) plates (24 g/L Potato Dextrose Broth, 20 g/L Agar) and incubated in a 30 °C static incubator for 5 days. The strains were further sub-cultured using Potato Dextrose Broth (PDB) medium and agar plates at regular intervals and stored at 4°C for preservation. The individual strains were streaked on PDA plates and incubated at 30°C for sporulation for 5 days. The spores were harvested using 0.9 % saline solution, stained using lactophenol cotton blue stain, and a hemocytometer was used for the determination of the concentration of spores of the different strains.

2.2 Materials

The chemicals used in this study were of analytical (AR) grade and were purchased from Hi-Media Laboratories, Mumbai (India). The disposed orange peels and apple pomace were collected from a fruit juice shop located outside the NIT Warangal campus.

2.3 Collection and drying of fruit waste

The orange peels and apple pomace were collected in bulk and cut down into smaller pieces for the ease of drying. They were then kept in a hot air oven (Laboratory Drying Oven, INSIF INDIA) at 50 °C with intermittent shaking for even drying, for 6 h, until a constant weight was achieved. The dried peels and pomace (Fig 2) were autoclaved and stored at – 20 °C for further experimentation.



Fig 2. Dried apple peels and pomace (left), and orange peels (right)

2.4 Screening of fungal strains

Four filamentous fungal strains, *A. fumigatus*, *A. terreus*, and two unidentified isolates (U1 and U2), isolated from NIT Warangal soil samples in the Bioprocess Development Laboratory during a previous study, were screened for pectinase production. Yeast Peptone Pectin (YPP) medium containing 10 g/L yeast extract, 20 g/L peptone, and 20 g/L pectin was prepared for screening of the pectinase enzyme production ability of the four fungal strains (KC *et al.*, 2020). 30 ml of YPP medium was inoculated with 1×10^6 spores of each fungal strain and incubated in a shaking incubator at 30 °C, 180 RPM for 11 days, based on a literature survey. Samples were collected at regular intervals and analyzed for pectinase activity using standard enzymatic methods (KC *et al.*, 2020). The *A. fumigatus*, being an opportunistic pathogen, was handled carefully following strict laboratory safety practices. The petriplates or media used for cultivation of the strain were decontaminated thoroughly, and the waste was disposed of in biohazard bins following institutional safety protocols.

2.5 Selection of the highest pectinase producer via SSF

Apple and orange peels were used as substrates for solid-state fermentation (SSF) to produce pectinase using fungal strains. Fungal spores were initially grown on a Potato Dextrose Agar (PDA) plate (24 g/L PDB, 20 g/L Agar) and harvested using 0.9% saline. An equivalent number of spores for the highest pectinase-producing strain was used to inoculate the apple and orange peels. Samples were collected at regular intervals for 11 days, and pectinase enzyme production was then estimated.

2.6 Optimization of pectinase production using Taguchi orthogonal array

Taguchi orthogonal array L9 (3^3) was employed for optimizing the production of pectinase using solid-state fermentation. The parameters selected for optimization—temperature (°C), pH, and solid-to-liquid ratio—were chosen based on a literature survey and experimental observations. The levels of these parameters are tabulated in Table 1.

Table 1. Levels of parameters used for the Taguchi orthogonal array

Parameters	Levels		
pH	5	6	7
Temperature (°C)	25	30	37
Solid:liquid ratio	1:1	1:2	1:3

Glass petriplates were used for the experiments (Fig 3), and samples were collected at 24 hr intervals for analysis. All experiments were performed in triplicate, and the mean values were used for further statistical analysis.



Fig 3. SSF for pectinase production using *A. fumigatus* using Taguchi orthogonal array.



The experimental data were analyzed using MINITAB statistical software (version 16, PA, USA). The experimental design used in this study has been tabulated in Table 2. The Signal-Noise (S/N) Ratio was used to assess the influence of different parameters on the pectinase enzyme production, with “Larger is better” selected as the quality characteristic. The S/N ratio was calculated using Equation 1:

$$S/N = 10 \log_{10} \left(\frac{\beta^2}{\alpha^2} \right) \quad (\text{Equation 1})$$

Where β is the mean (signal), and σ is the standard deviation (noise).

Table 2. L9 (3³) Orthogonal array of Taguchi experimental design for optimization of pectinase production using fruit peel as substrate

Sl. No	pH	Temperature (°C)	Solid/Liquid Ratio (S/L)	Pectinase Activity (U/ml)	S/N Ratio
P1	5	25	1:1	4.8 ± 0.007	13.62
P2	5	30	1:2	6.7 ± 0.019	16.52
P3	5	37	1:3	8.85 ± 0.022	18.93
P4	6	30	1:1	5.6 ± 0.015	14.96
P5	6	37	1:2	6.8 ± 0.004	16.65
P6	6	25	1:3	6 ± 0.020	15.56
P7	7	37	1:1	5.75 ± 0.006	15.19
P8	7	25	1:2	4.56 ± 0.014	13.17
P9	7	30	1:3	6.9 ± 0.01	16.77



The statistical significance of each parameter individually and in combination was analyzed using Analysis of Variance (ANOVA). The Mean Square Deviation (MSD), which measures the deviation from the target values, was calculated using Equation 2:

$$\text{MSD} = -10 \log_{10} \left[\frac{1}{n} \sum \frac{1}{Y_i^2} \right] \quad (\text{Equation 2})$$

Where **n** is the number of replications, and **Y_i** is the response (objective function). The optimal combination of parameter levels resulted in the maximum pectinase yield (U/ml).

2.7 Characterization of crude pectinase

Based on previous reports, the parameters pH, temperature, and the effect of metal ions were selected to characterize the crude pectinase enzyme produced by the selected fungal strain. These parameters were chosen to understand the enzyme's functional stability and to identify the optimal conditions for its maximum activity. The crude enzyme extract was diluted 100-fold to bring the enzyme concentration within a measurable range and to avoid substrate saturation during activity assays. The dilution ensured accuracy and consistency in the experimental results and allowed for reliable interpretation of the data.

2.7.1 Effect of pH

In order to optimize the pH required for the optimal activity of the crude pectinase enzyme, a 0.1 M Sodium citrate buffer was prepared in the following pH range: 3, 4, 5, 6, 7, and 8. The range was selected based on activity profiles of fungal pectinases reported throughout scientific literature. A reaction mixture was prepared by adding 200 µl of 1% pectin solution, dissolved in the respective pH buffer, and 100 µl of the diluted crude enzyme extract was added. The enzyme - substrate mixes were incubated for 30 mins and the activity was determined spectrophotometrically.

2.7.2 Effect of temperature

For the determination of the temperature required for the highest pectinase activity and thermal stability, similar experimental conditions as the pH optimization were maintained, with the temperature altered over the range 35 °C, 40 °C, 45 °C, 50 °C, 55 °C, and 60 °C. After a 30 min incubation period, the activity was measured spectrophotometrically.

2.7.3 Effect of metal ions

In attempts of understanding the effect of different metal ions on the crude pectinase activity, the optimized pH and temperature conditions were maintained. Different metal salts, including KCl, NaCl, MgCl₂, CaCl₂, CoCl₂, ZnCl₂, MnCl₂, FeCl₃, and CuSO₄, were added at a concentration of 50 mM. The activity was then measured spectrophotometrically after 30



minutes of incubation to assess the influence of each metal ion on pectinase activity. Different concentrations (10 – 100 mM) of the metal ion with the most profound effect on the pectinase activity were screened as well.

2.8 Analytical Methods

The samples collected from the submerged fermentation using YPP media were centrifuged at 11000 RPM for 5 min at room temperature using a tabletop centrifuge (Eppendorf). The supernatant was used for analyzing the pectinase activity. 0.5 g of fungal mash (fungus digested apple pomace or orange peel) was added to 5 ml of 0.1 M sodium citrate buffer. The mixture was manually crushed, vortexed, and centrifuged at 11000 RPM for 5 min at room temperature. The supernatant contained the crude pectinase enzyme. This was further diluted 100 times. 0.1 M sodium citrate buffer was used for preparing 1 % Pectin solution. 100 ml of the diluted crude enzyme was added to 200 ml of pectin, and the mixture was incubated for 30 min. After the incubation, 750 ml 3,5-dinitrosalicylic acid (DNS) reagent (Breuil & Saddler, 1985) was added to the mixtures immediately. The mixtures were then boiled in a water bath for 5 min and cooled down to room temperature. The absorbance was measured at 540 nm in a double-beam UV-visible spectrophotometer (Shimadzu) for analysis. The enzyme activity in this study is defined as the amount of pectinase enzyme required to produce 1 μ mol of galacturonic acid per minute under standard experimental conditions, expressed as U/ml. The pectinase enzyme activity throughout this study was calculated using the following formula (Equation 3).

$$\text{Units/ml of Pectinase Enzyme} = \frac{\text{Dilution factor} \times \mu\text{moles of product} \times \text{Volume of Assay}}{(\text{Time} \times \text{Volume of Enzyme})}$$

(Equation 3)

All experiments were performed using three independent replicates ($n = 3$), and the data are presented as mean $\pm$ standard deviation. A p -value < 0.05 was considered statistically significant. The standard deviation was shown as error bars in the graphical representation of the experimental data.

3. Results

3.1 Preparation of fruit waste

The apple pomace and orange peels were dried in the oven until the weight reduced to a constant value. The moisture contents of apple pomace and orange peels were found to be 11.25 % and 3.5%, respectively.



3.2 Screening of fungal strains for pectinase production

A standard curve was prepared using galacturonic acid for the estimation of the pectinase enzyme activity (Fig. 1). An R^2 value of 0.99 was obtained, establishing statistical significance. Among the four filamentous fungal strains screened, *Aspergillus fumigatus*, *Aspergillus terreus*, and two unidentified isolates (U1 and U2), *A. fumigatus* (GenBank: OQ618999.1) exhibited the highest pectinase activity of 4.4 U/ml when cultured in Yeast Peptone Pectin (YPP) medium (Fig 4). Enzyme activity was monitored at regular intervals over an 11-day incubation period at 30 °C, with the highest enzyme activity being observed on the 10th day of incubation. Based on this, *A. fumigatus* was selected for further optimization studies.

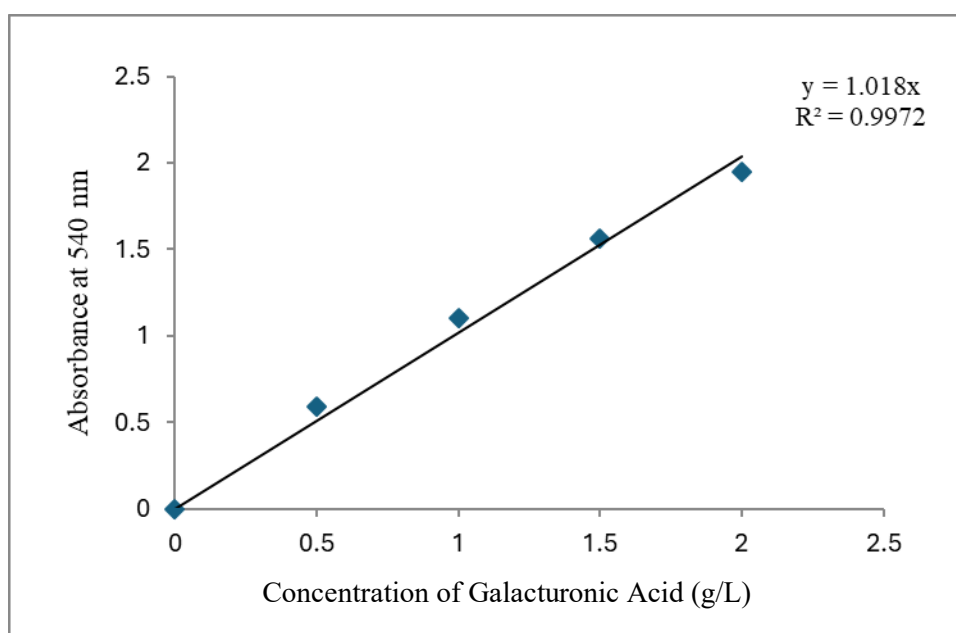


Fig. 1. Standard curve preparation using galacturonic acid.

3.3 Solid-state fermentation using Apple pomace and Orange Peel

The highest pectinase-producing strain *A. fumigatus* was cultivated under SSF using orange peel, apple peel, and pomace for the evaluation of the potential of the fruit wastes as suitable substrates for pectinase enzyme production. Samples were collected over 11 days at 24 hr intervals. Among the two tested substrates, orange peel supported significantly higher pectinase enzyme production by in comparison to apple pomace. A maximum pectinase activity of 4.44 U/ml was observed on the 10th day of the fermentation using orange peel, thus indicating its superiority as a substrate. A maximum pectinase activity of 1.6 U/ml was observed using apple pomace as substrate for SSF (Fig 5).

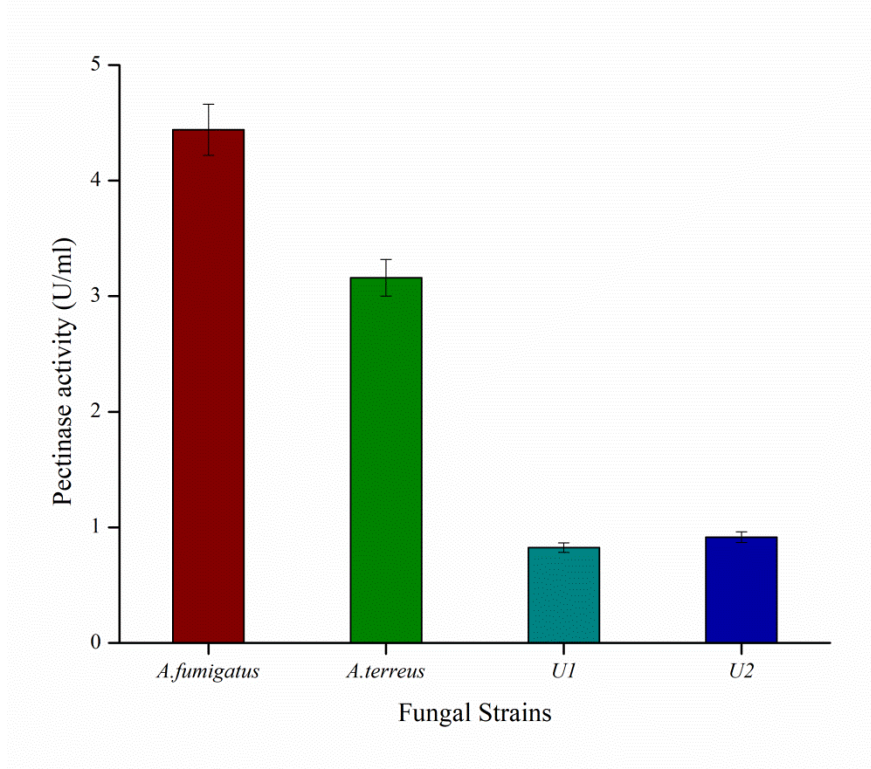


Fig. 4. Screening of fungal strains for pectinase production using YPP medium.

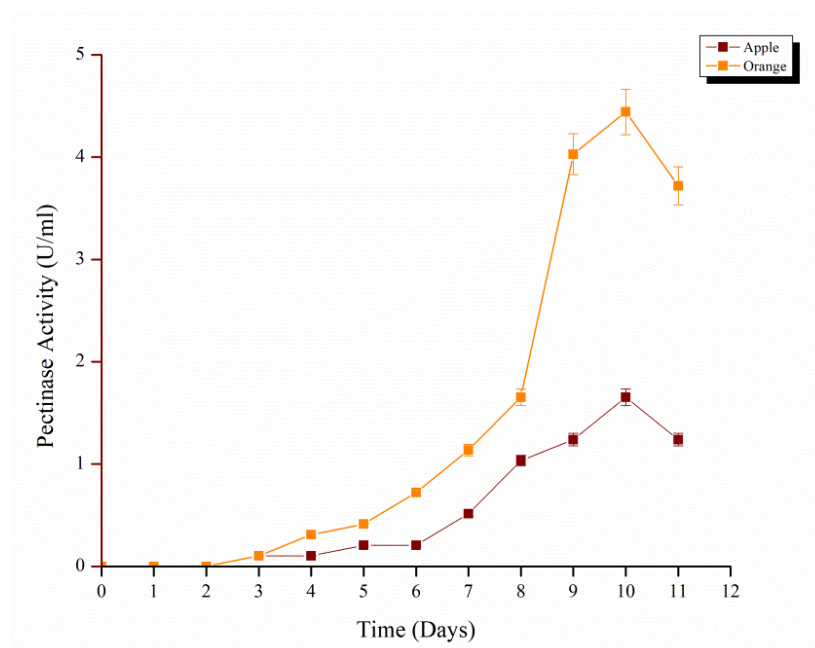


Fig. 5. Pectinase production by *A. fumigatus* on apple pomace and orange peel.



3.4 Optimization of pectinase production using Taguchi orthogonal array

The Taguchi orthogonal array was applied for the identification of optimal cultivation conditions for enhanced pectinase production using minimal experimental trials. Three independent variables were selected in three levels each - pH (5, 6, and 7), incubation temperature (25 °C, 30 °C, and 37 °C), and solid-liquid ratio (1:1, 1:2, and 1:3 w/v) to determine their influence on the pectinase enzyme production by *A. fumigatus*. 9 experimental runs were carried out based on the L9 array. The response - pectinase activity (U/ml) —was measured for each combination, and the data were analyzed using Signal-to-Noise (S/N) ratios and ANOVA to determine the effect of each parameter on the pectinase production (Table 2). The main effects of the parameters on the pectinase production have been represented in Fig 6.

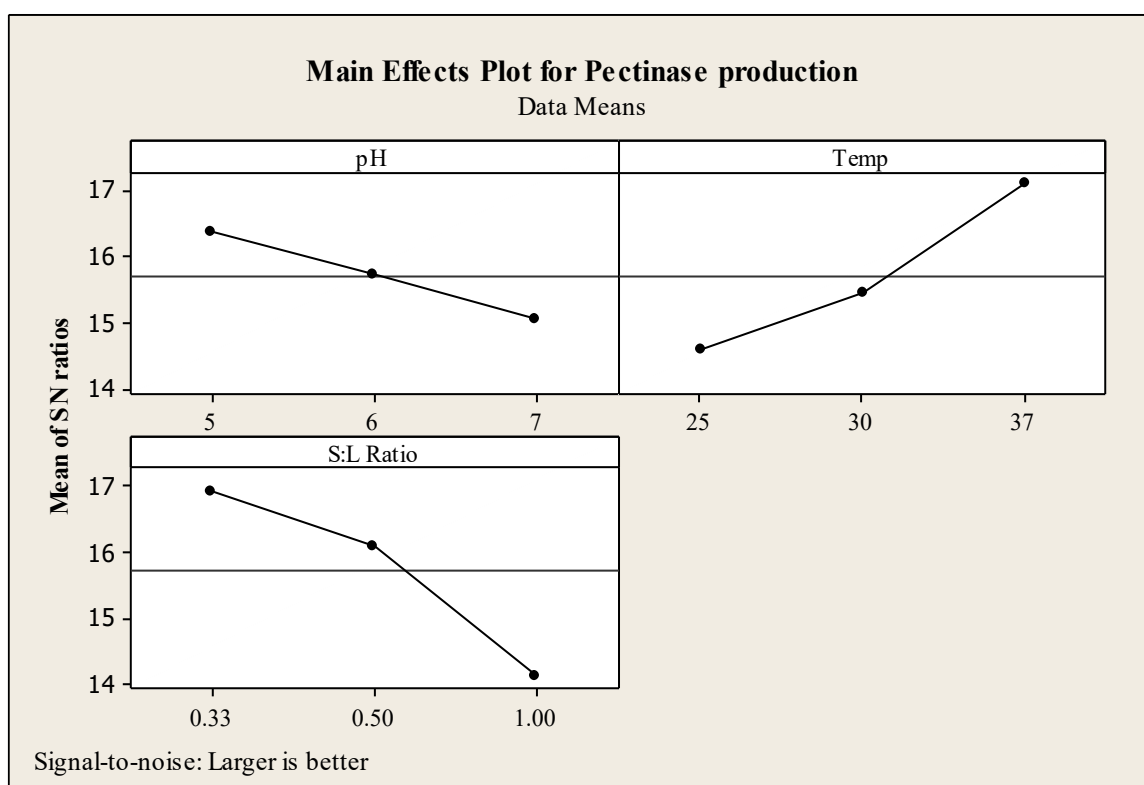


Fig. 6. Main effects plot for the parameters used in Taguchi orthogonal array for the optimization of pectinase production.



The interaction between the parameters chosen for optimization and the variance due to each was calculated using ANOVA. The model was considered to be statistically significant based on an F value of 96.44, and $p < 0.05$. In this study, the solid–liquid ratio had the highest delta S/N Ratio (2.81) and was designated as Rank 1, followed by incubation temperature (2.50), and then the pH of MSM (1.31). The parameters and levels are tabulated in Table 1. The coefficient of determination (R^2) was determined to be 98.3 %, indicating that the model fit the experimental data.

The regression equation for pectinase enzyme production was represented as Equation 4.

$$\text{Pectinase production (U/ml)} = 6.32 - 0.523 \text{ pH} + 0.157 \text{ Incubation temperature (}^\circ\text{C)} - 2.90 \text{ Solid - Liquid Ratio (w/v)}$$

(Equation 4)

The main effects plot (Fig 6) demonstrates the impact of the individual parameters on the pectinase production. The production of the enzyme was observed to be facilitated at a lower MSM pH of 5 and decreased with the increase of pH to a neutral range. Temperature seemed to have an opposite effect; enhancing the incubation temperature from 25 °C to 37 °C led to increased pectinase production. A lower solid-liquid ratio of 1:3 (w/v) led to a higher enzyme titer, which decreased thereafter. The maximum pectinase activity was observed in the optimized conditions of pH 5, temperature 37 °C, and a solid-liquid ratio of 1:3.

3.5 Characterization of crude pectinase enzyme

The crude pectinase enzyme produced by *A. fumigatus* using orange peel waste as a substrate was subjected to characterization studies to assess its behavior under different physico-chemical conditions.

3.5.1 Effect of pH on enzyme activity

Crude pectinase enzyme activity was assessed across a pH range of 3 to 8 using 0.1 M sodium citrate buffer. The maximum activity of 10.22 U/ml was observed at pH 7, and a decline was observed with further increase in pH (Fig 7a).

3.5.2 Effect of temperature on enzyme activity

The pectinase enzyme activity was measured at six different temperature points: 35 °C, 40 °C, 45 °C, 50 °C, 55 °C, and 60 °C, to identify the temperature required for enhanced enzyme activity, and 0.1 M sodium citrate buffer (pH 7) was used. Pectinase activity increased progressively from 35 °C and reached its peak at 45 °C, reporting 20.24 U/ml, beyond which a slight reduction in activity was noticed (Fig 7b).

3.5.3 Effect of metal ions on enzyme activity

Metal ions often serve as cofactors influencing the structural stability of the enzyme and enzyme - substrate interactions. A range of metal ions – NaCl, MnCl₂, FeCl₃, ZnCl₂, KCl,



CuSO₄, CaCl₂, MgCl₂, and CoCl₂ were tested at a concentration of 50 mM. The previously optimized experimental conditions – Citrate buffer with pH 7, and 45 °C temperature were maintained for all the reactions. Mg²⁺ exerted the maximum positive influence on the enzyme's catalytic potential, leading to a crude pectinase activity of 22.7 U/ml (Fig 7c). The effect of the Mg²⁺ ion concentration on the pectinase activity was further tested over a range of 10 mM – 100 mM, to identify the optimum. The pectinase enzyme exhibited the highest activity of 26.86 U/ml in the presence of 60 mM Mg²⁺ ions, indicating its significant effect on the enzyme stability and functioning (Fig 7d).

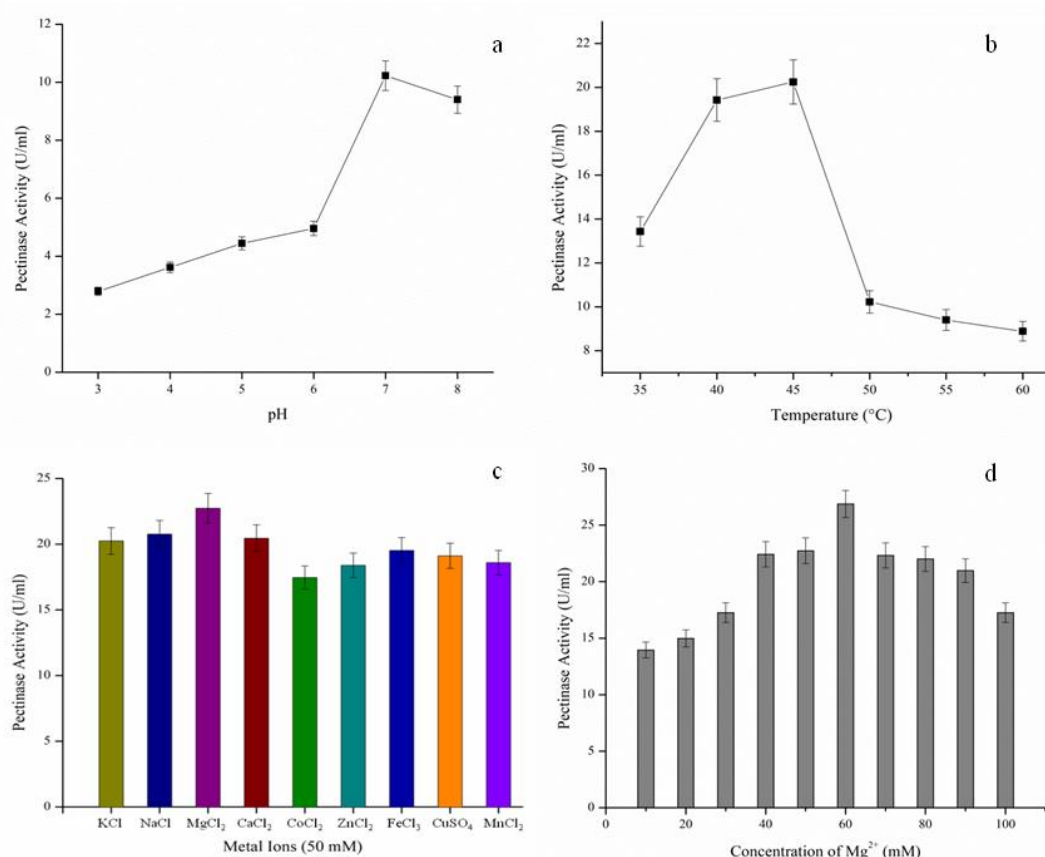


Fig 7. Characterization of crude pectinase; effect of (a) pH, (b) Temperature, (c) different metal ions, and (d) range of concentrations of Mg²⁺ on pectinase activity

4. Discussion

In the commercial enzyme market, the members of the *Aspergillus* genus are known for their prolific enzyme production abilities. Another study reported pectinase production by an *A. fumigatus* strain isolated from decomposing plant material (Okonji *et al.*, 2019). Other *Aspergillus* species like *A. niger* (Ahmed *et al.*, 2016), *A. oryzae* (Ketipally & Ram, 2018), *A.*



japonicus (Teixeira *et al.*, 2000), *A. awamori* (Botella *et al.*, 2007) have also been used for the production of pectinase enzyme using a range of natural pectin-rich substrates like orange peel and pomace, fruit wastes, banana waste, etc. Initially, a synthetic YPP medium containing pectin as the primary carbon source was used to identify the pectinase-producing abilities of the four strains used in this study. The pectinase activity of *A. fumigatus* consistently outperformed the other strains, indicating its superior potential for pectinase production under the tested conditions. In contrast, *A. terreus* and the two unidentified isolates showed comparatively lower enzyme yields. Usage of commercially available pectin as substrate for fermentation increases the net cost of the entire bioprocess, rendering it unsuitable for scale-up. The valorization of agro-industrial waste has gained momentum for sustainable enzyme production owing to the abundance and the low-cost. Hence, natural pectin rich fruit peels were considered, and apple peel and pomace, and orange peel were used as substrates for SSF for pectinase production using *A. fumigatus*. The concentration of pectin has been documented to vary considerably among different fruit peels, with citrus peels containing the highest (25 – 35 %), followed by apple peel and pomace (10 – 15%), sugarbeet (10 – 20%) etc. (Kumar *et al.*, 2020). Owing to the higher pectin concentration, orange peel as a substrate proved to be more suitable for pectinase production using *A. fumigatus*, leading to a titer of 4.44 U/ml on the 10th day of the SSF using orange peels. A study by Okonji *et al.* isolated an *A. fumigatus* strain from decomposing plant matter, which was also reported to produce a maximum of 3.52 U/ml pectinase enzyme via SSF using orange peel as substrate on the 6th day of fermentation (Okonji *et al.*, 2019). The maximum yield of pectinase enzyme produced by filamentous fungi via SSF of agro-industrial residues has been documented to be in the later stages of fermentation. The period of fermentation and the highest yield of product depend on a range of factors, like the nature of the fermenting strain, the physiological parameters of the media, as well as the nutrient availability (Patil & Dayanand, 2006). An *A. niger* strain produced the highest pectinase yield after the 5th day of SSF using orange peel waste (Ahmed *et al.*, 2016). Another study reported the highest pectinase enzyme titer produced by an *A. niger* on orange peel after the 3rd day of fermentation, however, sucrose and ammonium sulfate were supplemented as carbon and nitrogen sources (Mrudula & Murugammal, 2011). India, being the second largest producer of oranges, produces about 10,270,000 tonnes of fruit per year, out of which orange peel constitutes about 50 % of the fresh fruit weight (Suri *et al.*, 2022). A small fraction of this is employed as cattle feed, and the majority of it is disposed of via traditional crop-waste disposal methods, leading to further environmental burden (Laufenberg *et al.*, 2003). The utilization of this cheap and abundantly available resource as a substrate for enzyme production thus opens up multiple avenues for valorization, leading to a sustainable biorefinery establishment. The other substrate used in this study, apple peels and pomace, contained reducing sugars like glucose and fructose in abundance as compared to pectin, and the availability of monomeric sugars led to a possible shift in the fungal metabolism, thereby



resulting in reduced production of the pectinase enzyme. The availability of excess glucose in the medium led to the reduction in pectinase group of enzymes by a strain of *A. niger* (Fontana *et al.*, 2005), and an *A. japonicus* strain exhibited repression of endo- and exo-polygalacturonases (pectinases) when the medium contained high levels of glucose and sucrose along with pectin (Teixeira *et al.*, 2000), in accordance with the observations of this study.

Environmental parameters are known to significantly impact the production of fungal enzymes. Based on an extensive literature review, pH of the MSM, incubation temperature, and the solid to liquid ratio were selected for optimization via statistical design. The Taguchi orthogonal array was used for understanding the effect of these parameters on the pectinase enzyme production by *A. fumigatus*. The pH of the culture medium affects the enzyme secretion and stability, temperature influences the fungal metabolic activity and protein folding, and the solid-liquid ratio regulates moisture content and nutrient availability. Pectinase production by filamentous fungi has been documented to be favored in mildly acidic pH ranges. A study reported that maximum pectinase production occurred in an *A. oryzae* strain when the initial pH of the medium was 4, and enzyme titers dropped when the production medium pH was increased above 6 (Malvessi & Silveira, 2004), similar to the current study, where the optimum production was observed at pH 5, and decreased when the medium pH was increased further. Another *Aspergillus* spp. strain was documented to produce the highest pectinase titer at pH 5.8 (KC *et al.*, 2020). Adeleke *et al.* reported that pectinase enzyme, produced using orange peels by an *A. flavus*, an *A. oryzae*, and a *Penicillium atrovirens* strain, showed optimal production at pH 5.5, 5.5, and 5, respectively (Beris, 2012). The *A. fumigatus* strain used in this study was observed to produce the highest pectinase titer when the cultivation temperature was 37 °C. Literature suggests that the maximum yield of pectinase enzyme from the members of the *Aspergillus* genus has been observed when the cultivation temperature has been maintained in the range of 30 – 40 °C. An *Aspergillus* spp. strain produced the highest pectinase titer at 30 °C, and with increasing temperature, the enzyme production declined (KC *et al.*, 2020). In a study, an *A. niger* LFP-1 strain was reported to demonstrate the highest pectinase production at 30 °C and maintained closely similar yields at 37 °C and 40 °C, as well. The enzyme production decreased at lower (20 °C) and higher (50 °C) temperatures (Mat Jalil *et al.*, 2023). In the case of SSF, the moisture content plays an exceedingly important role, directly influencing the growth and enzyme production by filamentous fungi. Many studies have observed 60–65 % moisture content to be optimum for pectinase enzyme production using wheat grains and dried citrus peels, whereas 65 % was observed to be most suitable when deseeded sunflower heads were used as substrate (Patil & Dayanand, 2006). In this study, a higher moisture level of around 75 % (1:3 w/v solid–liquid ratio) was reported to lead to maximized pectinase production. The higher moisture contents of sugarcane bagasse (70%) and apple pomace (80%) have also



been documented to result in enhanced pectinase production (Patil & Dayanand, 2006). The *A. fumigatus* strain exhibited the maximum pectinase production with MSM of pH 5, incubation temperature 37 °C, and a solid-liquid ratio of 1:3. The statistical model was found to be significant, and further experimental validation of the optimized parameters confirmed the reproducibility and reliability of the Taguchi design.

The parameters pH of the buffer, reaction temperature, and the presence of certain metal ions exhibit significant roles in enhanced functioning of the enzyme by maintaining its structural integrity, and enhancing the active site – substrate binding (Das *et al.*, 2025).

The pH of the buffer impacts the enzyme activity greatly by influencing the ionization of amino acid residues in the active site, thereby affecting the substrate binding efficiency (Li *et al.*, 2022). The *A. fumigatus* crude pectinase showed a gradual increase in activity as the pH was increased from acidic to neutral. This suggested enhanced structural stability of the enzyme – substrate complex at neutral pH, ensuring effective interaction of the crude pectinase and pectin. At pH values lower than 7, reduced activity was observed, likely due to the destabilization of the enzyme structure, inactivation of the active site, or reduced substrate solubility. At pH 8, a slight decline in the enzyme activity indicated the enzyme's preference for a near-neutral environment rather than alkaline. Most pectinolytic enzymes have been reported throughout the literature to have the highest enzymatic activities in the slightly acidic pH range of 4–7. A pectinase enzyme from a *Thermomucor indicae-seudaticae* strain exhibited optimum activity at pH 5 when produced in submerged fermentation (SmF), and in the pH range 4–5, when produced in SSF conditions, and was reported to retain 80 % of its activity throughout the pH range 3–10, suggesting a wide range of pH tolerance of certain fungal enzymes (Martin *et al.*, 2010). Another study reported the pectinase enzyme produced by an *A. niger* in both SmF and SSF to be active in the pH range 3.5 – 5 (Acuña-Argüelles *et al.*, 1995). An *Aspergillus* spp. Gm strain was reported to show pectinase activity throughout a wide pH range of 3.2–9, showing the optimum activity at pH 5.8, and another distinct peak at pH 8 (KC *et al.*, 2020). An *A. niger* URM4645 strain was found to produce a range of pectinolytic enzymes using forage palm as substrate; the pH optima for endo-polygalacturonase and pectin esterase were observed at pH 5, and for exo-polygalacturonase at pH 7 (de Holanda Cavalcanti Maciel *et al.*, 2011), similar to the crude pectinase enzyme produced by the *A. fumigatus* strain.

Temperature acts as a crucial factor in enzymatic reactions by affecting the rate of molecular collisions, protein folding, and stability. The increase in the reaction temperature often leads to enhancement of the enzymatic activity due to the reduction of the activation energy (Das *et al.*, 2025). The enhancement of the crude pectinase activity up to 45 °C can be explained by an increase in the kinetic energy, which accelerates the frequency of the enzyme–substrate collisions. The observed optimum temperature suggests the stability of the enzyme in



moderate thermal conditions, making it a suitable candidate for biotechnological applications requiring higher temperatures. Beyond 45 °C, a slight decline in the enzyme activity was observed, possibly due to structural instability; however, complete denaturation and cessation of catalysis were not observed within the tested range. Similar temperature optima have been observed for pectinase enzymes produced by other filamentous fungi, with the endo-polygalacturonase, pectin esterase, and exo-polygalacturonase produced by *A. niger* URM4645 exhibiting maximum activities at 50 °C, 50 °C, and 45 °C, respectively (de Holanda Cavalcanti Maciel *et al.*, 2011). A pectin lyase enzyme produced by a *Penicillium oxalicum* in SSF using deoiled mandarin orange peel also reported maximum activity at 50 °C (Yadav & Shastri, 2007). The maximum activity for the pectinases produced by *A. fumigatus* TF3 and *A. terricola* was also reported to be at 50 °C, as reported by Phutela *et al.* (Phutela *et al.*, 2005) and Yadav *et al.* (Yadav *et al.*, 2009), respectively.

To further enhance the enzyme activity of the crude pectinase, a range of metal ions was tested. Metal ions often act as electrophiles or nucleophiles and impact the enzyme activity by altering the active site conformation and substrate availability. Based on specific enzyme active site structures and the amino acid residues present, different metal ions may exert enhancing or inhibitory effects. The Mg^{2+} ions were observed to have the greatest influence on the crude pectinase activity, which could either be via stabilization of the enzyme structure or participation in substrate availability. Other ions, including Ca^{2+} and Mn^{2+} , also showed positive effects on the enzyme activity, although less prominent compared to Mg^{2+} . This observation suggests that bivalent cations can potentially act as activators of the pectinase enzyme, with Mg^{2+} ions being the most effective for this specific crude *A. fumigatus* pectinase, under the tested conditions. A range of concentrations of Mg^{2+} was tested, and the enzyme activity was found to increase with the increase in concentration. The pectinase activity peaked when 60 mM Mg^{2+} ions were added to the reaction mix, and a decline in activity was observed upon further increase in ion concentration, possibly due to steric hindrance and enzyme structure modification (Das *et al.*, 2024; Secundo, 2013). The activity of the pectinase produced by *Penicillium italicum* was reported to be enhanced by Mg^{2+} , Ca^{2+} , and Mn^{2+} , in accordance with the observations reported in this study (Alaña *et al.*, 1990). The activity of a *P. chrysogenum* pectinase was also observed to be enhanced in the presence of Mg^{2+} and Ca^{2+} ions (Banu, 2010).



Table 3. Comparative study of pectinase enzyme production by filamentous fungi using fruit wastes.

Fungal strain	Substrate	Fermentation	Enzymatic Yield / Titer	References
<i>Aspergillus niger</i> Aa -20	Lemon peel pomace	SSF in column tray bioreactor (supplemented with Czapek – Dox medium)	2181 U/L Pectinase	(Ruiz <i>et al.</i> , 2012)
<i>Aspergillus awamori</i>	Mixture of grape pomace and orange peel	SmF in stirred tank bioreactor (supplemented with urea, ammonium sulphate, and mineral salts)	4 IU/g Exo-polygalacturonase	(Díaz <i>et al.</i> , 2012)
<i>Aspergillus niger</i>	Orange pomace	SmF (supplemented with ammonium sulphate and yeast extract 1:1)	17 U/g Endo-polygalacturonase 45 U/g Exo-polygalacturonase	(Mahmoodi <i>et al.</i> , 2017)
<i>Aspergillus parvisclerotigenus</i> KX928754	Apple pomace	Liquid static surface fermentation (supplemented with 2 % sucrose and 3 % peptone)	1366.30 U/ml Pectinase	(Satapathy <i>et al.</i> , 2021)
<i>Galactomyces candidum</i>	Mango pectin	SmF (supplemented with beef extract, and yeast extract)	39.96 U/mg Pectinase	(Ezugwu <i>et al.</i> , 2023)
<i>Aspergillus fumigatus</i>	Orange peel	SSF	8.85 U/ml Pectinase	(Present study)

A study by Khan *et al.* also documented the positive effect of a range of ions, especially Mg^{2+} , Ca^{2+} , and Mn^{2+} , among others, on the pectinase enzyme activity (Khan & Barate, 2016). Mg^{2+} and Ca^{2+} ions were observed to enhance the *Hylocereus polyrhizus* (Pitaya) peel pectinase activity by 112 % and 120 %, respectively (Amid *et al.*, 2014), and also enhanced



Bacillus subtilis pectinase activity when 4 mM concentration of ions were supplemented (Mahto *et al.*, 2023), thereby establishing the positive effect of the Mg^{2+} and Ca^{2+} ions on pectinase enzyme activities across different species including fungi, bacteria, as well as plants. A comparative study of pectinase enzyme production by filamentous fungi using fruit wastes as substrates has been tabulated in Table 3.

5. Conclusion

Pectinase, a commercially important enzyme, finds applications in food, agriculture, and textile industries. The production from agro-industrial residues presents significant economic and environmental advantages. The effective production of pectinase from orange peel mitigates fruit waste disposal issues and facilitates the development of valuable bio-products derived from abundant, renewable, and low-cost resources. A robust *A. fumigatus* strain, isolated from local soil, was reported to produce considerable titers of pectinase enzyme while growing on commercially available media and fruit peel wastes. This study aimed to identify the optimal conditions for the production of pectinase by optimizing key parameters, including pH, temperature, and solid-liquid ratio via Taguchi orthogonal array. The key findings indicated that the strain produced optimal titers of pectinase at pH 5, a temperature of 37 °C, and a solid-liquid ratio of 1:3. The characterization of the crude enzyme demonstrated the influence of different physicochemical factors on its activity. The enzyme exhibited the highest activity at pH 7 and at a reaction temperature of 45 °C. The incorporation of metal ions, specifically Mg^{2+} , significantly enhanced the enzyme's activity. The optimization of the process and parameters led to a net 510% increase in the crude pectinase activity. Pectinase enzyme is utilized in a plethora of industries for textile processing, paper manufacturing, fiber retting, etc. The high titers associated with the pectinase production from orange peel by the *A. fumigatus* strain can lead to the availability of an economic alternative for non-food industries. Further research is required for scaling up the enzyme production and decreasing the costs linked to the process. The stability of the crude pectinase enzyme under various storage and operational conditions, along with its potential applications in commercial processes, are some of the avenues that also need exploration before the establishment of a successful bioprocess. The effective production of pectinase from fruit peel waste indicates a significant advancement in the development of a sustainable bio-based economy, facilitating the transformation of agricultural residues into valuable products while promoting waste reduction and the production of value-added bioproducts.

Acknowledgment

The authors would like to acknowledge the Department of Biotechnology, NIT Warangal, for providing the necessary facilities to accomplish this research work. The funders had no role in the study design, data collection and analysis, publication decision, or article preparation.



Declarations

Ethical Approval: Not applicable

Competing interests: The authors declare that they have no competing interests.

Funding: Not applicable

6. References

- Abdollahzadeh, R., Pazhang, M., Najavand, S., Fallahzadeh-Mamaghani, V., & Amani-Ghadim, A. R. (2020). Screening of pectinase-producing bacteria from farmlands and optimization of enzyme production from selected strain by RSM. *Folia Microbiologica*, 65(4), 705–719. <https://doi.org/10.1007/s12223-020-00776-7>
- Abdullahi, H. Y., Kumar, M., Mishra, S. K., Dashora, K., Pandit, S., Saini, S., Tripathi, M., Mishra, P., Singh, A., Channashettar, V., Rajauria, G., & Kuhad, R. C. (2026). Spotlight on pectinase: a comprehensive review of large-scale production strategies. *Critical Reviews in Biotechnology*, 1–21. <https://doi.org/10.1080/07388551.2025.2587149>
- Acuña-Argüelles, M. E., Gutiérrez-Rojas, M., Viniegra-González, G., & Favela-Torres, E. (1995). Bioprocessing of citrus waste peel for induced pectinase production by *Aspergillus niger*; its purification and characterization. *Applied Microbiology and Biotechnology*, 43(5), 808–814. <https://doi.org/10.1007/BF02431912>
- Ahmed, I., Zia, M. A., Hussain, M. A., Akram, Z., Naveed, M. T., & Nowrouzi, A. (2016). Bioprocessing of citrus waste peel for induced pectinase production by *Aspergillus niger*; its purification and characterization. *Journal of Radiation Research and Applied Sciences*, 9(2), 148–154. <https://doi.org/10.1016/j.jrras.2015.11.003>
- Alaña, A., Alkorta, I., Domínguez, J. B., Llama, M. J., & Serra, J. L. (1990). Pectin Lyase Activity in a *Penicillium italicum* Strain. *Applied and Environmental Microbiology*, 56(12), 3755–3759. <https://doi.org/10.1128/aem.56.12.3755-3759.1990>
- Amid, M., Manap, Y., & Zohdi, K. (2014). Purification and characterisation of thermo-alkaline pectinase enzyme from *hylocereus polyrhizus*. *European Food Research and Technology*, 239(1), 21–29. <https://doi.org/10.1007/s00217-014-2188-x>
- Bamidele, M. O., Bamikale, M. B., Cárdenas-Hernández, E., Bamidele, M. A., Castillo-Olvera, G., Sandoval-Cortes, J., & Aguilar, C. N. (2025). Bioengineering in Solid-State Fermentation for next sustainable food bioprocessing. *Next Sustainability*, 6, 100105. <https://doi.org/10.1016/j.nxsust.2025.100105>
- Banu, A. R. (2010). Production and characterization of pectinase enzyme from *Penicillium chrysogenum*. *Indian Journal of Science and Technology*, 3(4), 377–381. <https://doi.org/10.17485/ijst/2010/v3i4.10>
- Beris, A. N. E. (2012). Production of Cellulase and Pectinase from Orange Peels by Fungi. *Nature and Science*, 6(5), 37–39.
- Botella, C., Diaz, A., de Ory, I., Webb, C., & Blandino, A. (2007). Xylanase and pectinase production by *Aspergillus awamori* on grape pomace in solid state fermentation. *Process Biochemistry*, 42(1), 98–101. <https://doi.org/10.1016/j.procbio.2006.06.025>
- Breuil, C., & Saddler, J. N. (1985). Comparison of the 3,5-dinitrosalicylic acid and Nelson-Somogyi methods of assaying for reducing sugars and determining cellulase activity.



- Enzyme and Microbial Technology*, 7(7), 327–332. [https://doi.org/10.1016/0141-0229\(85\)90111-5](https://doi.org/10.1016/0141-0229(85)90111-5)
- Das, S., Shakya, D., Kaur, R., B, N. K., T, C., Sevda, S., Selvasembian, R., & Prabhu, A. A. (2025). Application of Cellulase From Mutated *Aspergillus* sp. for the Production of Sustainable 2G Ethanol from Sugarcane Bagasse. *Bioenergy Research*, 18(1). <https://doi.org/10.1007/s12155-025-10825-z>
- Das, S., T, C., Selvasembian, R., & Prabhu, A. A. (2024). Mixed food waste valorization using a thermostable glucoamylase enzyme produced by a newly isolated filamentous fungus: A sustainable biorefinery approach. *Chemosphere*, 352, 141480. <https://doi.org/10.1016/J.CHEMOSPHERE.2024.141480>
- de Holanda Cavalcanti Maciel, M., Herculano, P. N., Porto, T. S., SimasTeixeira, M. F., Moreira, K. A., & de Souza-Motta, C. M. (2011). Production and partial characterization of pectinases from forage palm by *Aspergillus niger* URM4645. *African Journal of Biotechnology*, 10(13), 2469–2475. <https://doi.org/10.5897/AJB10.096>
- Díaz, A. B., de Ory, I., Caro, I., & Blandino, A. (2012). Enhance hydrolytic enzymes production by *Aspergillus awamori* on supplemented grape pomace. *Food and Bioproducts Processing*, 90(1), 72–78. <https://doi.org/10.1016/j.fbp.2010.12.003>
- Ezugwu, A. L., Onoyima, S. C., Eje, O. E., Nsude, C. A., Ugoh, A. I., & Chilaka, F. C. (2023). Properties and application of pectinase obtained from *Galactomyces candidum* using pectin extracted from mango peels as a carbon source. *Biocatalysis and Agricultural Biotechnology*, 51(May), 102763. <https://doi.org/10.1016/j.bcab.2023.102763>
- Fontana, R. C., Salvador, S., & Silveira, M. M. da. (2005). Influence of pectin and glucose on growth and polygalacturonase production by *Aspergillus niger* in solid-state cultivation. *Journal of Industrial Microbiology & Biotechnology*, 32(8), 371–377. <https://doi.org/10.1007/s10295-005-0004-0>
- Gawkowska, D., Cybulska, J., & Zdunek, A. (2018). Structure-Related Gelling of Pectins and Linking with Other Natural Compounds: A Review. *Polymers*, 10(7), 762. <https://doi.org/10.3390/polym10070762>
- Haile, S., & Ayele, A. (2022). Pectinase from Microorganisms and Its Industrial Applications. *The Scientific World Journal*, 2022, 1–15. <https://doi.org/10.1155/2022/1881305>
- Kaul, K., Rajauria, G., & Singh, R. (2024). Valorization of agro-industrial waste for pectinase production and its influence on circular economy. *Food and Bioproducts Processing*, 148, 141–153. <https://doi.org/10.1016/j.fbp.2024.09.008>
- KC, S., Upadhyaya, J., Joshi, D. R., Lekhak, B., Kumar Chaudhary, D., Raj Pant, B., Raj Bajgai, T., Dhital, R., Khanal, S., Koirala, N., & Raghavan, V. (2020). Production, Characterization, and Industrial Application of Pectinase Enzyme Isolated from Fungal Strains. *Fermentation*, 6(2), 59. <https://doi.org/10.3390/fermentation6020059>
- Ketipally, R., & Ram, M. R. (2018). Optimization of Pectinase Production by *Aspergillus oryzae* RR 103. *Current Agriculture Research Journal*, 6(1), 37–44. <https://doi.org/10.12944/carj.6.1.05>



- Khan, I. G., & Barate, D. L. (2016). Effect of Various Parameters on Activity of Pectinase Enzyme. *International Journal of Advanced Research*, 4(1), 853–862. <http://www.journalijar.com>
- Kumar, S., Ozukum, R., & Mathad, G. M. (2020). Extraction, characterization and utilization of pectin from apple peels. *Journal of Pharmacognosy and Phytochemistry*, 9(5), 2599–2604. <https://doi.org/10.22271/phyto.2020.v9.i5aj.12736>
- Laufenberg, G., Kunz, B., & Nystroem, M. (2003). Transformation of vegetable waste into value added products: *Bioresource Technology*, 87(2), 167–198. [https://doi.org/10.1016/S0960-8524\(02\)00167-0](https://doi.org/10.1016/S0960-8524(02)00167-0)
- Li, X., Zhao, R., Li, S., Wang, Y., Wang, X., Yang, W., Yang, M., Xiao, W., Yang, S., Lin, X., Zheng, X., Ma, X., Zhao, L., Xiao, W., & Cao, L. (2022). Global reprogramming of xylose metabolism in *Saccharomyces cerevisiae* efficiently produces ethanol from lignocellulose hydrolysates. *Industrial Crops and Products*, 179, 114666. <https://doi.org/10.1016/j.indcrop.2022.114666>
- Mahmoodi, M., Najafpour, G. D., & Mohammadi, M. (2017). Production of pectinases for quality apple juice through fermentation of orange pomace. *Journal of Food Science and Technology*, 54(12), 4123–4128. <https://doi.org/10.1007/s13197-017-2829-8>
- Mahto, R. B., Yadav, M., Muthuraj, M., Sharma, A. K., & Bhunia, B. (2023). Biochemical properties and application of a novel pectinase from a mutant strain of *Bacillus subtilis*. *Biomass Conversion and Biorefinery*, 13(12), 10463–10474. <https://doi.org/10.1007/s13399-021-02225-y>
- Malvessi, E., & Silveira, M. M. da. (2004). Influence of medium composition and pH on the production of polygalacturonases by *Aspergillus oryzae*. *Brazilian Archives of Biology and Technology*, 47(5), 693–702. <https://doi.org/10.1590/S1516-89132004000500004>
- Martens-Uzunova, E. S., & Schaap, P. J. (2009). Assessment of the pectin degrading enzyme network of *Aspergillus niger* by functional genomics. *Fungal Genetics and Biology*, 46(1), S170–S179. <https://doi.org/10.1016/j.fgb.2008.07.021>
- Martin, N., Guez, M. A. U., Sette, L. D., Silva, R. Da, & Gomes, E. (2010). Pectinase Production by a Brazilian Thermophilic Fungus *Thermomucor indicae seudaticae* N31 in Solid State and Submerged Fermentation 1. 79(3), 306–313. <https://doi.org/10.1134/S0026261710030057>
- Mat Jalil, M. T., Zakaria, N. A., Salikin, N. H., & Ibrahim, D. (2023). Assessment of cultivation parameters influencing pectinase production by *Aspergillus niger* LFP-1 in submerged fermentation. *Journal of Genetic Engineering and Biotechnology*, 21(1). <https://doi.org/10.1186/s43141-023-00510-z>
- Mrudula, S., & Murugammal, R. (2011). Production of cellulase by *Aspergillus niger* under submerged and solid state fermentation using coir waste as a substrate. *Brazilian Journal of Microbiology*, 42(3), 1119–1127. <https://doi.org/10.1590/S1517-83822011000300033>
- Okonji, R. E., Itakorode, B. O., Ovumedia, J. O., & Adedeji, O. S. (2019). Purification and biochemical characterization of pectinase produced by *Aspergillus fumigatus* isolated from soil of decomposing plant materials. *Journal of Applied Biology and Biotechnology*, 7(3), 1–8. <https://doi.org/10.7324/JABB.2019.70301>



- Oumer, O. J. (2017). Pectinase: Substrate, Production and their Biotechnological Applications. *International Journal of Environment, Agriculture and Biotechnology*, 2(3), 1007–1014. <https://doi.org/10.22161/ijeab/2.3.1>
- Patil, S. R., & Dayanand, A. (2006). Production of pectinase from deseeded sunflower head by *Aspergillus niger* in submerged and solid-state conditions. *Bioresource Technology*, 97(16), 2054–2058. <https://doi.org/10.1016/j.biortech.2005.09.015>
- Phutela, U., Dhuna, V., Sandhu, S., & Chadha, B. S. (2005). Pectinase and polygalacturonase production by a thermophilic *Aspergillus fumigatus* isolated from decomposting orange peels. *Brazilian Journal of Microbiology*, 36(1). <https://doi.org/10.1590/S1517-83822005000100013>
- Rashid, S. A., Dewa A. P., Dewi, R., Ibrahim, D., & Salikin, N. H. (2025). In-depth morphological characterization and identification of native fungal pectinase producer *Aspergillus niger* HFM 8 from rotten agro-wastes and soil environment. *Malaysian Journal of Microbiology*. <https://doi.org/10.21161/mjm.240826>
- Ruiz, H. A., Rodríguez-Jasso, R. M., Rodríguez, R., Contreras-Esquivel, J. C., & Aguilar, C. N. (2012). Pectinase production from lemon peel pomace as support and carbon source in solid-state fermentation column-tray bioreactor. *Biochemical Engineering Journal*, 65, 90–95. <https://doi.org/10.1016/j.bej.2012.03.007>
- Sajish, S., Tomar, G. S., Singh, S., & Kaushik, R. (2025). A low-cost and sustainable approach to microbial pectinase production from fruit processing wastes: from peel to profit. *Environmental Technology*, 1–18. <https://doi.org/10.1080/09593330.2025.2555498>
- Satapathy, S., Soren, J. P., Mondal, K. C., Srivastava, S., Pradhan, C., Sahoo, S. L., Thatoi, H., & Rout, J. R. (2021). Industrially relevant pectinase production from *Aspergillus parvisclerotigenus* KX928754 using apple pomace as the promising substrate. *Journal of Taibah University for Science*, 15(1), 347–356. <https://doi.org/10.1080/16583655.2021.1978833>
- Secundo, F. (2013). Conformational changes of enzymes upon immobilisation. *Chemical Society Reviews*, 42(15), 6250. <https://doi.org/10.1039/c3cs35495d>
- Siddiqui, M. A., Pande, V., & Arif, M. (2012). Production, Purification, and Characterization of Polygalacturonase from *Rhizomucor pusillus* Isolated from Decomposting Orange Peels. *Enzyme Research*, 2012, 1–8. <https://doi.org/10.1155/2012/138634>
- Suri, S., Singh, A., & Nema, P. K. (2022). Current applications of citrus fruit processing waste: A scientific outlook. *Applied Food Research*, 2(1), 100050. <https://doi.org/10.1016/j.afres.2022.100050>
- Teixeira, M. F. S., Lima Filho, J. L., & Durán, N. (2000). Carbon sources effect on pectinase production from *Aspergillus japonicus* 586. *Brazilian Journal of Microbiology*, 31(4). <https://doi.org/10.1590/S1517-83822000000400009>
- Yadav, S., & Shastri, N. (2007). Purification and properties of an extracellular pectin lyase produced by the strain of *Penicillium oxalicum* in solid-state fermentation. *Indian Journal of Biochemistry & Biophysics*, 44, 247–251.
- Yadav, S., Yadav, P. K., Yadav, D., & Yadav, K. D.S. (2009). Purification and Characterization of Pectin Lyase Produced by *Aspergillus terricola* and its Application in Retting of Natural Fibers. *Applied Biochemistry and Biotechnology*, 159(1), 270–283. <https://doi.org/10.1007/s12010-008-8471-1>