Research Article

Molecular Identification of Pectinolytic Bacteria Isolated from Rotten Fruit and Optimization of Pectinase Production Using Response Surface Methodology

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Abstract | Pectinases are enzymes of major industrial interest. This study aimed to isolate and characterize pectinolytic bacteria obtained from decomposing fruit and optimize the composition of the culture medium. Bacteria were isolated on a reconstituted, selective pectin-enriched medium. Active colonies were characterized by morphological observations, biochemical tests, and molecular identification of 16S rDNA. Of the 55 isolates selected, 12 (21.81%) were pectinolytic. All isolates were Gram-positive, predominantly bacillary, and positive for the citrate test. Five strains exhibited γ-haemolysis, indicating that they were non-haemolytic. Based on 16S rRNA gene sequencing, four of these bacteria that expressed high pectin degradation indices were related to *Lactiplantibacillus plantarum*, *Acetobacter tropicalis*, *Bacillus amyloliquefaciens*, and *Bacillus subtilis*. The *B. amyloliquefaciens* strain isolated from the decomposing cashew apple, which expressed the highest pectinolytic index (3.659) and had a non-haemolytic profile, was selected for optimizing its pectinase production. Unifactorial optimization of pectinase production by this strain showed that pectin was the most favourable carbon source, $(NH_4)_2HPO_4$ was the best nitrogen source, and the optimum pH for activity was 7. Multifactorial optimization using four independent factors showed that the quadratic model optimized the yield by 160-fold, with a maximum activity of 19.3953 IU/ml at 32.50 °C, and pH of 7 in a buffered medium. Among the variables studied, pectin concentration appeared to be one of the most influential factors.

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Introduction

Pectinases are a heterogeneous group of enzymes of technological interest that degrade pectic polysaccharides in the plant cell walls (de Souza and Kawaguti, 2021; Jin *et al.*, 2024). Their classification is based on their mode of action on pectin. Thus, these enzymes are classified into three groups (Murad and Azzaz, 2011). First, the protopectinases, or pectinases, which hydrolyze the insoluble protopectin into a highly polymerized soluble pectin. On the other hand, the enzymes that modify the structure of pectin include both the pectin methylesterases that demethylate the methoxyl groups, and the depolymerization enzymes, such as pectin lyases, pectate lyases, polygalacturonases and polymethylgalacturonases, which catalyze the trans-elimination lysis and hydrolytic cleavage of the glycosidic bonds in the pectic substances (Murad and Azzaz, 2011). These enzymes are present in plants, microorganisms, nematodes, and insects and express broad substrate specificity (de Souza and Kawaguti, 2021). They are therefore considered promising biocatalysts for all processes that require pectin hydrolysis (Hailé and Ayele, 2022). In the fruit and vegetable juice industry, these pectinases improve clarification, stability, and extraction yield, reduce viscosity, and enhance organoleptic qualities (Sharma *et al.*, 2017; Alqahtani *et al.*, 2022; Haile and Ayele, 2022; Sheladiya *et al.*, 2022). They also play a role in the processing of coffee, tea, cocoa, and tobacco by facilitating fermentation, mucilage removal, and release of bioactive compounds and aromas (Oumer and Abate, 2017; Samanta, 2019; Mulluye and Atnafu, 2022). Their applications also extend to the extraction of oils and plant compounds, animal feed, valorization of agri-food waste, bioethanol production, paper processing and the textile industry, where they used in combination with cellulase to improve the cotton fibers (Anand *et al.*, 2020; Ruchika *et al.*, 2020; de Souza and Kawaguti, 2021; Shrestha *et al.*, 2021; Khan *et al.*, 2025). A previous study demonstrated antitumor effects of pectinases on colorectal and endothelial cells (Cho *et al.*, 2019). These enzymes account for approximately 25% of food enzymes sold and used worldwide, and their demand continues to increase (Jayani *et al.*, 2005; Amin *et al.*, 2019). Most commercially available pectinases are of fungal origin and industrial production began in 1970 (Haile and Ayele, 2022). Although fungal pectinases are the most studied and the main source of these enzymes, their production can present challenges, including relatively slow growth, sometimes complex nutritional requirements, and, in some cases, the co-production of undesirable secondary metabolites such as mycotoxins and allergenic compounds, which may complicate the purification and increase the production costs (Geris *et al.*, 2024). These challenges, combined with the growing demand for industrial pectinases, justify exploring the new microbial sources. In this context, bacteria represent a promising alternative because they exhibit rapid growth and extracellular enzyme secretion, thereby simplifying recovery and reducing production costs (Ramin and Allison, 2019). Furthermore, they can adapt to a wide range of conditions, covering a broad spectrum of pH and temperatures. Furthermore, bacteria are biological systems that are generally more amenable to strain improvement approaches, particularly through selection, mutagenesis, or genetic engineering, thereby increasing enzyme production. Their tolerance to phenolic inhibitors, combined with their shorter generation time compared to fungi, also allows for faster evaluation of the effects of multiple culture parameters (Sui *et al.*, 2025). This speed offers advantages in the application of multifactorial optimization methods, such as response surface methodology (RSM), to evaluate the influence of the interactions between several factors and identify the optimal production conditions. Available studies focused primarily on the enzymatic activity and optimization of pectinase production conditions (Jalil and Ibrahim, 2021; Alqahtani *et al.*, 2022; Blanco Crivelli *et al.*, 2024; Satpathy *et al.*, 2025; Panchal *et al.*, 2026). However, these studies rarely addressed the preliminary safety assessment of the producing strains, particularly through investigating their hemolytic activity, even though this aspect is important for considering their use in food applications. The decaying tropical fruits, such as cashew apples, remain relatively unexplored as potential niches for pectinase-producing bacteria. The cashew apples natural state of decomposition could be exploited to isolate microorganisms capable of degrading cell-wall polysaccharides, especially pectin. In this context, this study aimed to explore the decomposition of cashew apples as a potential local source of the pectinolytic bacteria. It combined screening for enzymatic activity, preliminary safety assessment of isolates, and statistical optimization of pectinase production conditions in a high-performing, non-hemolytic strain. This approach could help identify the bacterial strains of interest for future biotechnological applications.

Materials and Methods

Isolation and screening of the pectinolytic bacteria

Bananas, mangoes, oranges, cashew apples, and soumbala, a condiment made from fermented *Parkia biglobosa* seeds, served as matrices for isolating the pectinolytic bacteria. For each type of fruit, a single composite sample was created from healthy fruit randomly collected from 5 to 7 vendors in different markets in Ouagadougou, the capital of Burkina Faso (Figure 1). The fruit was then stored for 21 d at room temperature until visible signs of spoilage appeared, before being used for the isolation of pectinolytic bacteria. Soumbala, being a fermented product, was used directly after its random collection from 5 vendors. Stock solutions were prepared by mixing 10 g of each sample into 90 ml of 0.9% (w/v) NaCl, followed by performing serial dilutions. A 100 µl volume of each dilution was inoculated onto a reconstituted culture medium containing pectin as the sole carbon source, according to the method described by Priyanka and Prajna (2019). The reconstituted culture medium, MGR medium (Medium growth receptor) solution was composed of 10 g pectin, 0.1 g $MgSO_4$, 3 g K_2HPO_4, 2 g KH_2PO_4, 5 g NaCl, 3 g $(NH_4)_2HPO_4$, and 25 g agar per 1 l of dist. water, and the pH was adjusted to 6. The plates were incubated at 37 °C for 96 h (Priyanka and Prajna, 2019). After incubation, all the developed bacterial colonies were purified by successive sub-culturing onto a pectin agar medium. The purified isolates were stored at 4°C in nutrient broth (NB) supplemented with 15% glycerol for further studies. To detect the pectinase activity, pure bacterial isolates were inoculated by spot deposition on the surface of MGR agar and incubated at 37 °C for 24 h. After incubation, the plates were covered with iodine/ potassium iodide (I_2/KI) solution (0.1% w/v) to identify the development of transparent halo around the colonies, indicating that the bacteria hydrolysed the pectin (Mercimek Takcı and Turkmen, 2016). The pectin degradation index (PDI) (Equation 1) was calculated as described by Haile and Kang (2019). Bacterial isolates exhibiting a halo around the colonies were noted for the haemolysis test.

$$PDI = \frac{\text{Diameter of clear zone} - \text{Diameter of bacterial colony}}{\text{Diameter of bacterial colony}} \quad \ldots(1)$$

Haemolysis test

Columbia agar (OXOID Ltd, PB pH 7.5 ± 0.2, Wesel, Germany) supplemented with 5% (V/V) sheep blood (CA-SB) was used to evaluate the haemolytic capacity of the isolates. Spot inoculation was performed, and incubation was carried out at 37°C for 48 h (Lee *et al.*, 2023). The formation of a clear or green halo around the bacterial colonies indicated β-haemolytic or α-hemolytic activity, respectively. Isolates without a halo around the colonies were regarded as γ-hemolytic (Kavitha *et al.*, 2018) and were considered non-haemolytic.

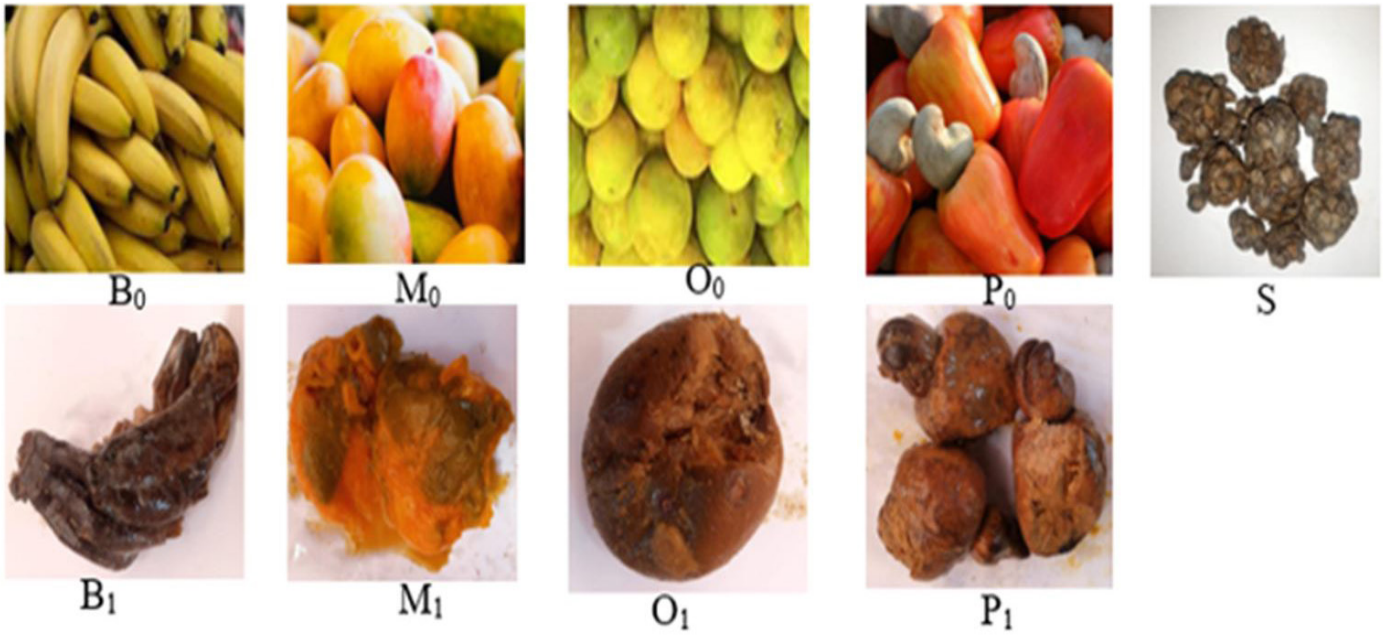

Figure 1: *Samples used for the isolation of pectinase-producing bacteria. B_0: Fresh bananas, B_1: Rotten bananas, M_0: Fresh mangoes, M_1: Rotten mangoes, O_0: Fresh Oranges, O_1: Rotten oranges, P_0: Fresh Cashew apples, P_1: Rotten cashew apples, S: Soumbala.*

Morphological and biochemical characterization of the bacterial isolates

Studies on the colony morphology, biochemical and molecular analyses were conducted on the isolates, which were selected based on their pectinolytic index. Morphological and biochemical analyses were performed according to the approach described by Shrestha *et al*. (2021), while molecular identification was conducted in accordance with the methodologies used for bacterial identification by *16S rRNA* gene analysis (Janda and Abbott, 2007). The color, shape, and size of each colony were recorded. For each selected isolate, various biochemical assays were carried out including the catalase test, the indole and Voges Proskauer (VP), gas production from glucose and mannitol, Ortho Nitro Phenyl Galactopyranoside (ONPG), arginine dihydrolase (ADH), lysine decarboxylase (LDC), ornithine decarboxylase (ODC), tryptophan deaminase (TDA), hydrogen sulphide (H_2S) production, use of citrate, and the gelatinase assay, according to Shrestha *et al*. (2021).

Molecular characterization of the bacterial isolates

Each bacterial isolate was cultured on pectin agar and incubated at 37°C under aerobic conditions for 24 h. Genomic DNA was rapidly extracted from a bacterial colony by heat shock, as described by Dashti *et al*. (2009). The extracted DNA was amplified by PCR, and the resulting amplicons were subjected to Sanger sequencing using the chain-termination method described by Sanger *et al*. (1977). This service included PCR amplification of the *16S rRNA* gene, targeting the region encoding the 30S subunit of the bacterial ribosome. The variable regions V1 to V5 were specifically targeted to distinguish among the bacterial populations by aligning the sequences against an international reference database. PCR was performed using genomic DNA from each bacterial isolate and two primer pairs. The first pair, composed of P8 (5′-AGAGTTTTGATCCTTGGCTCAG-3′) and P535 (5′-GTATTACCGCGGCTGCTGGCAC-3′), and the second pair, 338-1040F (5′-CTCCTACGGGAGGCAG-3′) and 338-1040R (5′-GACACGAGCTGACGACA-3′), amplified fragments of approximately 550 bp and 750 bp, respectively. These primers targeted the universally conserved regions of the *16S rRNA* gene, and the resulting fragments were used to identify each isolate molecularly. The PCR conditions included 40 cycles of denaturation, hybridization, and elongation, with positive and negative controls. Amplicons were verified by agarose gel electrophoresis and QIAxcel analysis (QIAGEN). The molecular weights of the amplified DNA fragments were calculated using size markers ranging from 50 to 1500 bp (QXDNA size marker, QIAGEN). After purification of the PCR products on a membrane (Macherey-Nagel), the amplicons were quantified by a fluorimetry with SYBR Green, using an Infinite® 200 microplate reader (Tecan, Switzerland). Sequencing of the 16S rRNA gene was carried out using a purified PCR product, with a minimum quantity of approximately 50 ng per reaction, corresponding to a concentration of 10-20 ng/µl. Afterward, the amplicons were sequenced in both directions using the same primers, according to an optimized protocol. The resulting sequencing reactions were purified on a Sephadex-G50 gel (GE Healthcare) and loaded onto an ABI 3730XL capillary sequencer.

Analysis and processing of the bioinformatic data

The raw sequences were optimized using Auto Peak Trace 6 RP software (Nucleics, Australia) and assembled into contigs using a Sequencher V4.9 software (Gene Codes Corporation, USA). To determine the phylogenetic affiliation of the isolates, the resulting 16S rRNA genes were compared by BLASTn against those of other bacterial strains available in the GenBank at NCBI, using the BLASTn1 option (Zhang *et al*., 2000) and the specialized EZbiocloud2 for bacterial 16S rRNA sequences (Yoon *et al*., 2017). The resulting sequences were manually checked and corrected using a BioEdit version 7.2 software. Sequence alignment was conducted using a MEGAX software with the Muscle algorithm (Kumar *et al*., 2018).

Unifactorial study of the components of the pectinase production medium

The *B. amyloliquefaciens* (PC25p) strain isolated from the decomposing cashew apple was selected for the optimization of pectinase production because it had the highest pectinolytic index (3.659) among the tested isolates and showed no haemolytic activity on blood agar. The effect of different growth factors on pectinase production by the selected bacterium was evaluated using the approach described by Satpathy *et al*. (2025).

Influence of different carbon sources: Pectinase production was carried out in 100 ml of broth medium in 250 mL Erlenmeyer flasks. The base culture

medium contained K_2HPO_4, KH_2PO_4, $MgSO_4$, and NaCl to which a different carbon source (pectin, glucose, sucrose, or arabinose) was added individually at different concentrations (5.00, 10.00, and 15.00 g/l), as along with $(NH_4)_2HPO_4$ as a nitrogen source. The pH of the medium was adjusted and maintained at 6. The broth medium was inoculated with 5% (v/v) of a bacterial suspension standardized to an optical density of 0.6 at 600 nm and then incubated under shaking at 200 rpm at 37 ± 2 °C for 3 d. The activity of the pectinases was then determined, where its methodology will be described later under the title "Determination of pectinase activity", and the carbon source inducing the highest activity was selected for subsequent studies.

Influence of different nitrogen sources: A protocol similar to the previous one was followed to identify the optimal nitrogen source among the various nitrogen compounds. The tested nitrogen sources included yeast extract, peptone, and $(NH_4)_2HPO_4$ were added individually at different concentrations (2.00, 3.00, and 5.00 g/l) to a basal medium containing pectin as the sole carbon source and buffered at pH 6. The activity was determined after 3 d of incubation with shaking.

Influence of pH: Bacterial cultures were grown individually in culture media adjusted to different pH levels using appropriate buffers: citrate-phosphate buffer for pH 2, 3, 4, 5, and 6; phosphate buffer for pH 7; and Tris-HCl buffer for pH 8 and 9. The culture medium was formulated using the previously determined optimal carbon and nitrogen sources. The cultures were incubated with shaking for 3 d. After incubation, the broth cultures were centrifuged at 4427 g for 30 min. at 4 °C. The resulting supernatant, corresponding to the enzymatic fraction, was used to evaluate the pectinase activity.

Multifactorial optimization of pectinase production

Statistical optimization of the growth factors influencing pectinase production in the selected *B. amyloliquefaciens* (PC25p) was performed using a centred composite design to increase the enzyme yield (Govindaraji and Vuppu, 2020). This design is a valuable tool in response surface methodology for improving the enzyme yield. This type of experimental design allows modelling of the linear, quadratic, and interaction effects of the factors under study, while reducing the number of trials required. The factors selected for optimization included pectin, $(NH_4)_2HPO_4$, temperature, and K_2HPO_4, which were selected based on preliminary trials. Coded levels were assigned to each factor according to the structure of the centred composite design, including factorial, axial, and central points. Design-Expert 7.0 software was used to design the experimental trials. A total of 30 trials combining different values of the four factors were designed and then carried out experimentally in the laboratory as shown in Table 1. Following the trials, the enzyme activity (expressed in IU/ml) was measured and used as a response variable to assess optimization of pectinase production. The experimental response was fitted to a second-order polynomial model to establish the relationship between the pectinase activity and the independent variables studied. The following equation (Equation 2) generated by the Expert Design software version 7.0.0 describes the regression model used in the factorial planning, including the interaction terms:

$$Y = a_0 + \sum_{i=1}^{n} a_i X_j + \sum_{j=1}^{n=1} \sum_{j=2}^{n} a_{ij} X_i X_j + \sum_{i=1}^{n} a_{ii} X_i^2 \ldots (2)$$

In this equation, Y represents the response predicted by the model, n corresponds to the number of factors studied, x_i and x_j represent the independent variables. The term a_0, corresponds to the constant of the model; while a_i, a_{ii}, and a_{ij} denote respectively the coefficients of the linear, quadratic, and interaction effects. The significance and goodness of fit were assessed using analysis of variance (ANOVA). All three-dimensional and contour plots, as well as the statistical analysis were performed using Design-Expert 7.0 software.

Table 1: *Coded levels and actual values of the independent variables.*

Indépendant variable	Levels		
	-1	0	1
Pectin (g /l)	5	9.62	15
$(NH_4)_2HPO_4$(g/l)	2	3.65	4
Temperature (°C)	25	31.09	40
K_2HPO_4(g/l)	2	2.83	4

Determination of pectinase activity

At the end of each culture, the different pectinase production broth media used with *B. amyloliquefaciens* (PC25p), both during single-factor and multi-factor optimization trials, were centrifuged at 4427 g for 30 min. at 4 °C. The supernatants obtained were collected

and used as crude enzyme extracts, containing the extracellular pectinases produced by the selected bacteria isolate. The pectinase activity of each crude extract was then determined using the 3,5-dinitrosalic acid (DNS) colorimetric method described by Miller (1959) with minor modifications. This method was based on quantifying the reducing sugars released during hydrolysis of the pectic substrate by the pectinases. The reaction medium consisted of 100 µl of crude enzyme extract and 900 µl of 1% pectin dissolved in 50 mM citrate-phosphate buffer, pH 5. The mixture was incubated with stirring at 30 °C for 5 min. Enzyme activity was stopped by adding 1000 µl of 3,5- dinitrosalicylic acid (DNS). Afterward, the reaction mixture was heated in a water bath at 100 °C for 5 min. Substrate blanks were also prepared under the same conditions as the enzymatic tests, replacing the enzyme extract with the buffer. The absorbance of the colored complex formed after reaction with DNS was measured at 540 nm using a Biotek Instruments Inc. Epoch™ spectrophotometer (Highland Park, Winooski, VT 05404-0998, USA). Enzyme activity was estimated from a calibration curve established using galacturonic acid at concentrations ranging from 0 to 0.05 mM. The resulting linear regression equation

$$y=75.757\ X+0.2254;\ R^2=0.9984\ \ldots\ldots\ (3)$$

allowed determination of the amount of galacturonic acid equivalents released during pectin hydrolysis. One unit of pectinase activity is defined as the amount of enzyme required to release 1 µmol of galacturonic acid equivalent per minute under the experimental conditions (Qureshi *et al.*, 2012). Enzyme activity (A) in IU/ml of the crude enzyme extract was calculated following this formula (Equation 4) (Shrestha *et al.*, 2021):

$$A = \frac{C\,.Vr}{T.Ve} D \quad \ldots(4)$$

Where; A: pectinase activity; C: Concentration of galacturonic acid determined from the standard curve and expressed in µmol/ml; Vr: Reaction volume in ml; Ve: Volume of crude enzyme extract in ml; D: Dilution factor.

Statistical analyses

One-way ANOVA was performed using XLSTAT software version 2016.02.27444 at a significance level of $\alpha = 0.05$ ($p < 0.05$). In cases of significant differences between the studied parameters, the means were ranked using the Newman-Keuls method. RSM was used to determine the optimal interactions among the factors and estimate the optimal conditions for pectinase production with the minimum number of experiments (Kottilingal *et al.*, 2026). RSM determines the effects of the independent variables on the processes, either individually or collectively. A central composite design comprising 30 experiments was applied for the statistical analysis. The experiments were performed in biological/experimental triplicate. For determination of the pectinolytic index, each isolate was subcultured independently onto three separate Petri plates. For the broth cultures, the PC26p isolate was cultured in three independent tubes, maintained under the same experimental conditions. A single measurement was performed for each independent culture and the results were expressed as mean ± standard deviation (±SD). For the analysis of variance, RSM, and the determination of the regression equations and the optimal parameters, Expert Design software version 7.0.0 was used.

Results and Discussion

Screening of the pectinase-producing bacterial isolates

Soumbala and four rotten fruits were analyzed to determine the presence of pectinolytic bacteria. A total of 145 different isolates (Table 2) cultured on reconstituted medium were obtained based on their color, elevation, consistency, transparency, and outline. After purification, 55 of these were selected and subjected to a pectin hydrolytic activity test by immersing their culture plates in I_2/KI solution. Of the 55 isolates, pectin degradation that was indicated through the formation of a clear halo was observed in approximately 12 bacterial isolates, representing 21.81%. Bananas and cashew apples yielded the most pectinolytic isolates, with 4 isolates each. Oranges yielded three isolates, while rotten mangoes produced the fewest isolates, only one. In contrast, no pectinolytic isolates were obtained from soumbala (Table 2). The variability observed in the distribution of pectinolytic isolates can be explained by the physicochemical differences in the matrices and ecological conditions. The high number of pectinolytic strains observed in bananas and cashew apples could be attributed to their higher levels of pectins and fermentable sugars. The availability of pectic substrates in these two matrices could thus promote the proliferation of the pectinolytic bacteria

Table 2: *Origins and morphological characteristics of the isolated pectinase-producing bacterial isolates.*

Samples	Strain code	Isolated strains	Isolates with a halo	Clear zone	Shape	Grouping method	Color	Size
Banana	B1 à B25	25 (17.24)	B6p	+	Stick	Isolated	Whitish	Medium
			B16p	+	Shell	Isolated	Whitish	Medium
			B20p	+	Shell	Isolated	Yellowish	Medium
			B24p	+	Shell	Isolated	Whitish	Small
Mango	M1 à M30	30 (20.68)	M29p	+	Oval	Isolated	Whitish	Large
Orange	O1 à O30	30 (20.68)	O5p	+	Stick	Isolated	Yellowish	Small
			O25p	+	Stick	Chain stitch	Whitish	Large
			O30p	+	Shell	Chain stitch	Whitish	Large
Cashew apple	PC1 à PC30	30 (20.68)	PC10p	+	Oval	Isolated	Yellowish	Large
			PC22p	+	Shell	Chain stitch	Whitish	Medium
			PC25p	+	Shell	Chain stitch	Whitish	Small
			PC26p	+	Stick	Isolated	Whitish	Medium
Soumbala	S1 à S30	30 (20.68)	-	-	-	-	-	-
Total	-	145 (100%)	12 (21.81%)	-	-	-	-	-

Where; B, O, M, PC, and S designate bacterial isolates obtained from bananas, oranges, mangoes, cashew apples, and soumbala, respectively. The isolates are numbered B1 to B25, O1 to O30, M1 to M30, PC1 to PC30, and S1 to S30 according to their origin. The letter p placed after the number indicates isolates exhibiting pectinolytic activity.

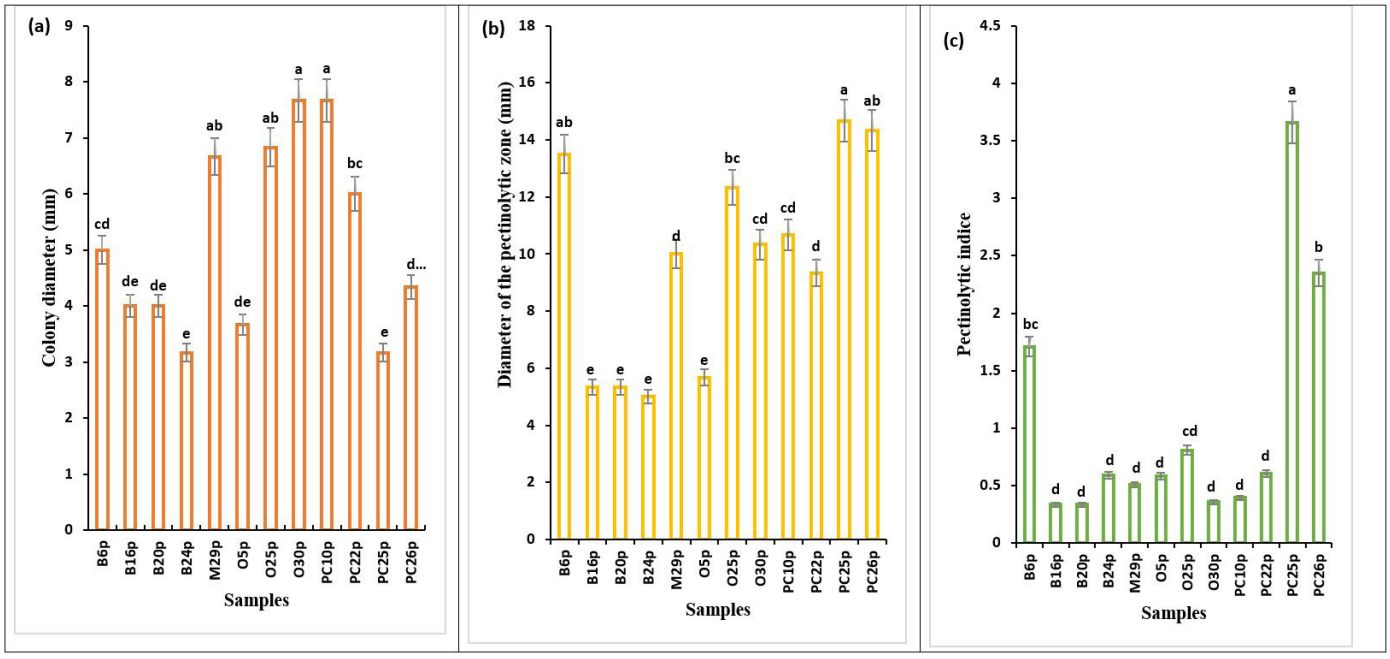

Figure 2: *Pectinolytic potential of the isolated bacteria. Diameters of pectin hydrolysis zones (a), colony diameters (b), and pectin degradation indices (c) of the 12 isolated bacterial isolates. Values are presented as mean ± standard deviation (±SD) over three determinations. Different letters above the bars indicate significant differences among the isolates for each parameter ($p < 0.05$).*

(Aguila and Huitron, 1987). Conversely, the low number of isolates observed in mangoes and oranges may be linked to the acidic pH of these matrices and/or the presence of inhibitors that inhibit the bacterial growth (Shaik and Chakraborty, 2022; Kučuk *et al.*, 2024). The complete absence of isolates in soumbala can be explained by the nature of this product, which is produced by alkaline fermentation. The pH and substances produced during fermentation are not favourable to the pectinolytic bacteria (Sheladiya *et al.*, 2022). Several recent studies involving the screening of pectinolytic bacteria have reported substantial numbers of these microbial species in the various environments (Oumer and Abate, 2018;

Alqahtani *et al*., 2022; Owusu *et al*., 2024). The overall rate of 21.81% that we obtained was higher than that reported previously by Umar *et al*. (2023). However, higher rates than ours have been reported by Aaisha and Barat (2016); Shrestha *et al*. (2021) for forest soil and coffee pulp samples, with rates reached of 58.6% and 51.4%, respectively. These differences could be assigned to the nature of the isolation matrix, stage of sample decomposition, environmental conditions, or the composition of the isolation medium. The addition of the KI solution highlighted clear areas of varying diameters around the 12 selected isolates, as shown in Figure 2a. The diameters ranged from 5 mm to 14.67 mm. The most extensive hydrolysis zones were observed around isolates PC25p and PC26p obtained from rotten cashew apples. These values are lower than those reported by Ghazala *et al*. (2016); Shrestha *et al*. (2021), who reported hydrolysis zones reaching 21 mm and 17 mm in diameter, respectively, for some isolates. However, lower values than ours were reported by Kamalambigeswari *et al*. (2018). The smallest pectinolytic zone diameters were observed with isolates from bananas. Although this matrix allowed for the isolation of many isolates, the hydrolysis diameters were small, ranging from 5 mm to 5.33 mm. This clearing zone observed around the colonies can be explained by the bacterial pectinases hydrolysing the pectin, thereby preventing the formation of an iodine complex (Shrestha *et al*., 2021). Regarding the sizes of the different colonies shown in Figure 2b, they varied from 3.16 to 7.66 mm (reflecting different growth rates between the isolates). The greatest bacterial growth was observed in isolate O30p, followed by isolates PC10p and O25p. The smallest colony diameter (3.16 mm) was recorded in isolate B24p. Determining the pectin degradation index is a commonly used approach to assess the ability of the isolates to hydrolyze pectin. In the present study, the analyzed isolates showed pectinolytic indices varying statistically from 0.33 to 3.65 (Figure 2c), reflecting temarkable variability in their pectinolytic activities. The best pectin degradation indices were obtained with isolates PC25p (3.65), PC26p (2.35), and B20p (1.70). The lowest indices were obtained with isolates B6p (0.33), B16p (0.33), and O30p (0.35). This heterogeneity observed among the isolatess can be attributed to the intrinsic differences among them and the affinity of their pectinases for pectin, which was used as the sole carbon source. Furthermore, the degradation index was widely used in the literature to quantify the enzymatic activity due to its simplicity and ability to provide a rapid estimate of bacterial pectinolytic activity. However, it remains a semi-quantitative estimation (Okonji *et al*., 2019; Mohammed *et al*., 2021). Thus, the highest pectinolytic indices observed for isolates PC25p and PC26p revealed that these two isolates exhibited the highest pectinase activity. These results suggest that food matrices such as cashew apples, bananas, oranges, and decomposing mangoes could be used to isolate the pectinase-producing bacteria.

Phenotypic characteristics

Biochemical characterization revealed a diversity of metabolic profiles (Table 2). All isolates were Gram-positive and tested positive for citrate, demonstrating their ability to utilize alternative carbon sources. However, all isolates tested negative for hydrogen sulphide, urease reduction, lysine decarboxylase, and indole (Table 3). All isolates tested positive for glucose utilization. Some isolates also tested positive for rhamnose, sucrose, and arabinose. This explains the isolates' ability to utilize a wide variety of carbohydrates as a carbon source. The study of the assimilation of various carbon sources by different isolates indicated that the pectinolytic iolates had a high capacity to assimilate mono- and disaccharides, as well as complex sugars. Asmaa *et al*. (2020) found similar results to ours. Their analysis showed that sucrose and fructose were the most frequently used sugars by the bacterial isolates, followed by glucose. All isolates were unable to assimilate lysine, as confirmed by the negative LDC (lysine decarboxylase) test. However, some isolates were positive for ornithine decarboxylase (ODC). Furthermore, all isolates were positive for tryptophan deaminase (TDA). These results indicated that the current isolates can assimilate these amino acids as a nitrogen source. Similar trends were reported by Kaur *et al*. (2016), who observed positive results for TDA. Some isolates were positive for Gelatinase, indicating that they were capable of hydrolyzing the gelatin and therefore possessed gelatinase enzyme. Furthermore, all isolates were catalase-positive, indicating their ability to tolerate oxidative stress. However, they all showed negative results for ortho-nitrophenyl-β-galactopyranoside (ONPG) and urea. These results indicated the inability of these isolates to hydrolyze these two compounds, which were substrates for urease and β-galactosidase. β-galactosidase is an inducible enzyme and its synthesis depends on the presence of lactose in the medium and its entry into the bacterial cells. Its absence could be explained by an

Table 3: *Biochemical and molecular characteristics of the bacterial isolates exhibiting pectinolytic properties.*

Tests conducted	Isolated pectinolytic bacteria											
	B6p	**B16p**	**B20p**	**B24p**	**M29p**	**O5p**	**O25p**	**O30p**	**PC10p**	**PC22p**	**PC25p**	**PC26p**
Gram	+	+	+	+	+	+	+	+	+	+	+	+
Mobility	-	-	+	-	-	+	-	-	+	-	-	-
ONPG	-	-	-	-	-	-	-	-	-	-	-	-
ADH	-	-	-	-	-	-	-	+	-	+	-	-
LDC	-	-	-	-	-	-	-	-	-	-	-	-
ODC	-	-	+	-	+	-	-	-	-	-	-	+
Citrate	+	+	+	+	+	+	+	+	+	+	+	+
H_2S	-	-	-	-	-	-	-	-	-	-	-	-
Ureate	-	-	-	-	-	-	-	-	-	-	-	-
TDA	+	+	+	+	+	+	+	+	+	+	+	+
Indole	-	-	-	-	-	-	-	-	-	-	-	-
V-P	+	+	+	+	+	-	+	-	-	+	+	+
Gelatinase	-	+	-	-	+	-	-	+	+	+	+	-
Glucose	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol	-	+	+	-	+	-	+	-	+	+	+	-
Inositol	-	+	-	-	+	-	-	+	+	+	+	-
Sorbitol	-	+	-	-	+	-	-	+	+	+	+	-
Rhamnose	-	-	-	-	+	-	-	-	-	+	-	-
Sucrose	-	+	-	+	+	-	-	+	+	+	-	-
Melibiose	-	-	-	-	+	-	-	+	-	+	+	-
Amygdaline	-	+	-	-	+	-	-	+	+	+	-	-
Arabinose	-	-	+	-	-	-	+	-	-	-	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+	+

Identified species

Strain	Identified species	GenBank accession number of deposited sequences	Similarity (%)	Top-hit strain	Number base pairs (pb)	Habitat
B20p	*Lactiplantibacillus plantarum*	PQ845133	99.91	*CP103911.1*	1069	Banana
O25p	*Acetobacter tropicalis*	PQ845142	100.00	*KJ469773.1*	992	Orange
PC25p	*Bacillus amyloliquefaciens*	PQ845326	99.90	*HQ850702.1*	1043	Cashew apple
PC26p	*Bacillus subtilis*	PQ845369	99.71	*KP676117.1*	1050	Cashew apple

Where; ONPG= Ortho nitrophenylgalactopyranoside; ADH= arginine dihydrolase; LDC= Lysine decarboxylase; ODC= ornithine decarboxylase; H_2S= Hydrogen sulfide; TDA= Tryptophan desaminase; V-P= Vogue-Proskauer; B=Banana; O= Orange; PC= Cashew apple.

inability to utilize lactose due to the lack of transport. Contrary to our results, Umar *et al.* (2023) reported positive results for *B. thuringiensis* isolated from soil, with the ONPG and urea tests suggesting genetic diversity among the isolates.

Haemolytic activity

Figure 3a shows the distribution of isolates by haemolytic activity. Among the 12 isolates tested on blood agar, seven isolates namely B6p, B16p, B24p, M29p, O5p, O30p, and PC22p showed a clear halo around the colonies, indicating β-haemolysis, representing 58.33% of the isolates. This reflects the isolate's ability to lyse the blood cells. Conversely, five isolates specifically B20p, O25p, PC10p, PC25p, and PC26p showed no halo zone; thus, they were considered γ-haemolytic. The absence of a halo around these colonies indicated that they were unable to cause erythrocyte lysis. Haemolytic activity is an essential criterion for assessing the safety of bacteria of interest to the food industry (Nwagu *et al.*, 2020). Haemolysis, whether partial (α) or complete (β),

is an indicator of virulence. Indeed, β-haemolysis reveals the presence of cytotoxic phospholipases in the bacteria and indicates that the haemolytic factor reduces the amount of haemoglobin available as an iron source for the host cells (Sorokulova *et al.*, 2008).

Molecular characteristics of the pectinase-producing bacterial isolates

The potential isolates for pectinase production were identified by *16S rRNA* gene analysis. The sequencing results were analyzed and compared to probable sequences in NCBI. In the present study, sequencing of the 16S rRNA gene and sequence comparison revealed similarity rates ranging from 99.71% to 100% with the type species in NCBI (Table 4). Isolate B20 exhibited a maximum identity of 99.91% with *Lactiplantibacillus plantarum* (OZ064365.1). As for isolates O25p, PC25p, and PC26p, they exhibited maximum identity of 100%, 99.90%, and 99.71%, with *Acetobacter tropicalis* (DQ523494.1), *B. amyloliquefaciens* (CP054415.1), and *B.s subtilis* (KF626465.1), respectively (Table 5). Of the molecularly identified isolates, approximately 75% belonged to the genus *Bacillus*. According to Priest (1977), the pectinolytic activity is widespread in the genus *Bacillus*. Some studies have also shown that many strains of this genus produce pectinase (Roosdiana *et al.*, 2013; Oumer and Abate, 2018). Furthermore, during the screening, isolates PC25p and PC26p, which secreted the highest amounts of pectinase, were also member of the genus *Bacillus*. This result is consistent with those of Oumer and Abate (2018), who reported that *Bacillus* sp. can produce large amounts of extracellular pectinase.

The phylogenetic tree was constructed using the closest sequences from NCBI GenBank with *E. coli* as the outgroup. The phylogenetic analysis indicated that several isolates exhibited a marked evolutionary proximity to known species. Isolate B20p (PQ845133) was phylogenetically related to *Lactiplantibacillus plantarum*, O25p (PQ845142) to *Acetobacter tropicalis*, PC25p (PQ845326) to *B. amyloliquefaciens*, and PC26p (PQ845369) to *B. subtilis*. However, species-level identification, particularly for those isolates belonging to the genus *Bacillus*, should be considered provisional, due to the high similarity of 16S rRNA sequences among the closely related species. These affiliations are presented in Table 3 and illustrated by the phylogenetic tree (Figure 3b). This diversity is consistent with data from the literature, which reports a wide distribution of the pectinolytic microorganisms in the natural environments rich in the organic matter (Haile and Ayele, 2022). These species observed in our isolates differ from those reported by Abdel-Aziz *et al.* (2019), who identified *Klebsiella oxytoca* from the soil samples. Similarly, Abdollahzadeh *et al.* (2020) isolated *Enterobacter* species from the fields. Furthermore, fruit waste dumps yielded *B. tropicus* (Thakur *et al.*, 2021). Along the same lines, *B. cereus*, *Streptomyces* sp. and *Aspergillus niger* isolated from fruits, vegetables, and soil samples were reported by Kh *et al.* (2022). The observed differences between

Table 4: *Matrix of the 4-factor central composite design and response function values.*

Run	X_1	X_2	X_3	X_4	DO	Activity (UI/ml)	Predicted value
1	20	3	32.5	3	0.96	19.3953	11.87
2	10	3	32.5	3	0.7	12.5306	11.74
3	15	2	25	4	0.56	8.8343	11.33
4	5	2	25	2	0.58	9.3623	8.91
5	10	3	32.5	3	0.57	9.0983	11.74
6	0	3	32.5	3	0.33	2.7617	5.89
7	15	4	40	2	0.36	3.5537	4.80
8	10	3	32.5	1	0.75	13.8508	10.64
9	15	2	40	2	0.29	1.7056	4.69
10	10	3	32.5	3	0.72	13.0587	11.74
11	10	5	32.5	3	0.48	6.7221	5.36
12	5	4	25	4	0.46	6.194	6.67
13	10	3	47.5	3	0.23	0.1214	-2.30
14	15	4	25	4	0.79	14.9069	15.40
15	10	3	32.5	5	0.72	13.0587	11.87
16	10	1	32.5	3	0.61	10.1544	7.12
17	10	3	32.5	3	0.79	14.9069	11.74
18	15	4	40	4	0.32	2.4976	6.40
19	15	4	25	2	0.59	9.6264	12.87
20	10	3	32.5	3	0.67	11.7386	11.74
21	15	2	40	4	0.28	1.4415	3.26
22	5	4	25	2	0.41	4.8739	4.00
23	5	2	40	4	0.45	5.93	6.14
24	5	2	40	2	0.49	6.9861	7.44
25	10	3	17.5	3	0.61	10.1544	8.17
26	15	2	25	2	0.55	8.5702	11.84
27	10	3	32.5	3	0.57	9.0983	11.74
28	5	4	40	4	0.44	5.666	3.34
29	5	2	25	4	0.56	8.8343	8.54
30	5	4	40	2	0.25	0.6495	1.61

X1: Pectin (g/l); X2: $(NH_4)_2HPO_4$ (g/l) (g/l); X3: Temperature (°C); X4: (K_2HPO_4) (g/l), DO: Optical density

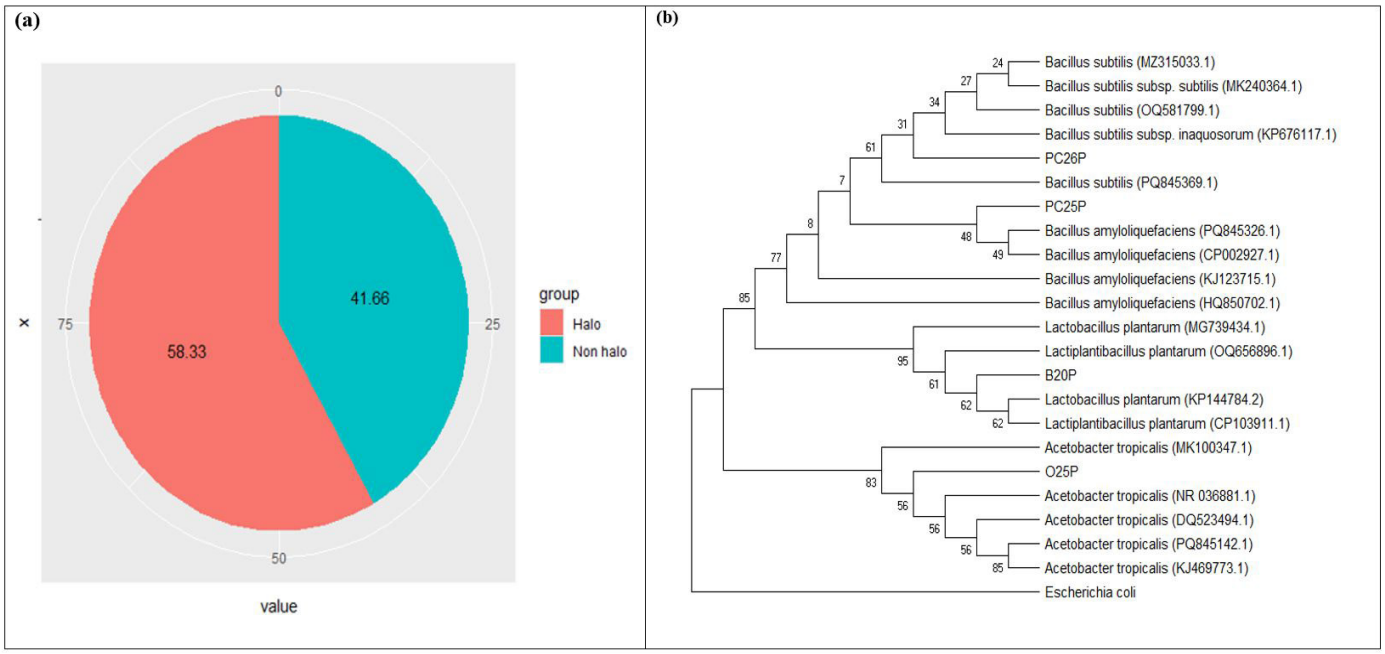

Figure 3: *Haemolytic profile and phylogenetic identification of the bacterial isolates selected based on their pectinolytic potential. (a) Distribution of the 12 isolates according to their haemolytic profile), (b) phylogenetic tree of the four best pectinase-producing strains.*

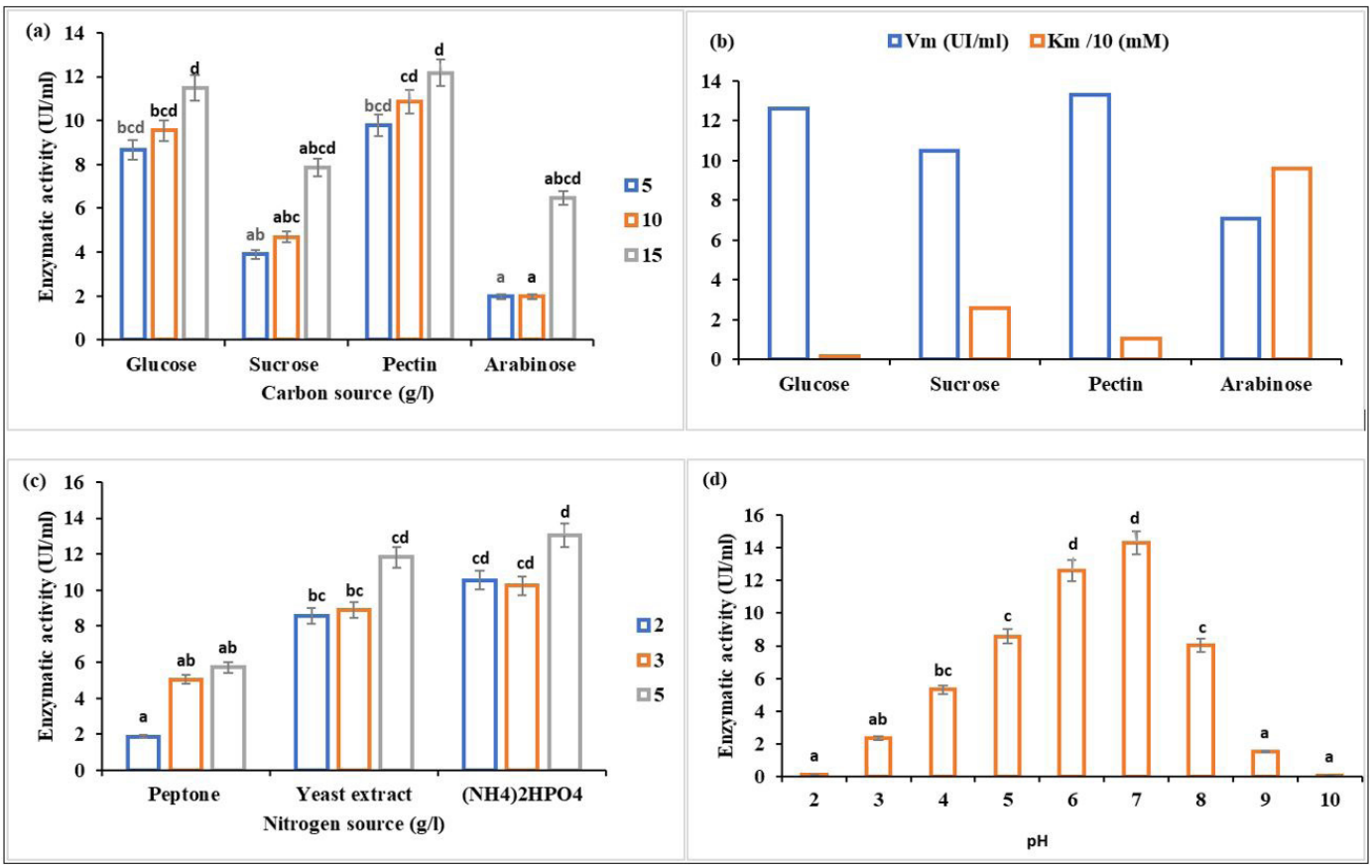

Figure 4: *Single-factor optimization of parameters influencing pectinase production by the Bacillus amyloliquefaciens-related strain. (a) Effect of carbon source on enzyme production, (b) Estimated values of the kinetic parameters Vmax and Km of pectinase, (c) Effect of nitrogen source and (d) initial pH of the medium on pectinase activity. Values are presented as mean ± standard deviation (±SD) (n=3). For subfigures (a), (c), and (d), the different letters above the bars indicate statistically significant differences among the treatments at the 5% level ($p < 0.05$). In subfigure (b), Km and Vmax are expressed in different units; therefore, they are presented only as kinetic parameters and should not be interpreted as comparable values.*

our strains and those reported in the recent studies can be attributed to variations in the ecological niches as well as the physicochemical parameters of the isolation environment. Despite this diversity, these microorganisms share the same ability to degrade pectin. The strain PC25p exhibiting the best pectin degradation index, which was γ-haemolytic, was therefore selected to study the optimal conditions for its pectinase production using a one-factor design and a centred composite design.

Table 5: *Analysis of variance (ANOVA) for optimizing the pectinase enzyme production efficiency based on the central composite design source.*

	Pectinase activity			
Source	**Sum of squares**	**Degree of freedom**	**Mean square**	**P > F**
Model	473.61	14	33.83	0.0344*
X_1-Pectin	53.72	1	53.72	0.0571*
X_2- $(NH_4)_2H_pO4$	4.65	1	4.65	0.5536
X_3-Temperature	164.53	1	164.53	0.0026*
X_4-K_2H_pO4	2.28	1	2.28	0.6774
X_1 X_2	35.29	1	35.29	0.1157
X_1 X_3	32.22	1	32.22	0.1314
X_1 X_4	0.017	1	0.017	0.9709
X_2 X_3	0.85	1	0.85	0.7986
X_2 X_4	9.22	11	9.22	0.4068
X_3 X_4	0.85	1	0.85	0.7986
X^12	14.02	1	14.02	0.3091
X_2^2	51.87	1	51.87	0.0611
X_3^2	132.78	1	132.78	0.0055*
X_4^2	0.40	1	0.40	0.8610
Residuel	189.81	15	12.65	
Lack of fit	163.46	10	16.35	0.1117**
Pure error	26.35	5	5.27	
Cor total	663.43	29		

Optimization of pectinase production by Bacillus amyloliquefaciens PQ845326 using a single factor design

The effects of different carbon and nitrogen sources and pH on pectinase production were investigated. The results showed a variation in enzyme activity across the different media compositions. As shown in Figure 4a, maximum production was obtained when pectin was used as the sole carbon source, followed by glucose, and then sucrose. Conversely, the lowest activities were obtained with arabinose. These results differed from those recently reported by Sukmawati *et al.* (2025), who found low activities with pectin and higher activities with sucrose in yeast pectinases. Furthermore, pectinase activity increased with increasing the carbon source concentration. For all the tested four carbon compounds, the highest activities were observed at 15 g/l, which was the maximum concentration evaluated in this study. Also Figure 4b presents the kinetic parameters (Vm and Km) of the pectinase under study with respect to the various substrates, including glucose, sucrose, pectin, and arabinose. Variations were observed depending on the substrate, reflecting the marked enzymatic specificity. Pectin exhibited the highest Vm value (13.31 UI/ml), indicating a higher maximum reaction rate and thus greater catalytic efficiency of the pectinase enzyme on this substrate. Furthermore, its relatively low Km value reflected good enzymatic affinity, confirming that pectin was the enzyme's preferred substrate. Glucose displayed a high Vm but a very low Km (1.32 mM), suggesting a high apparent affinity, although it was not the primary natural target of the pectinases, reflecting its nonspecific interactions. Sucrose showed intermediate values for Vm (10.46 UI/ml) and Km (25.8 mM), indicating moderate catalytic efficiency and affinity. In contrast, arabinose had the lowest Vm (7.07 UI/ml) along with the highest Km (96 mM), indicating its low catalytic efficiency and very low enzyme affinity for this substrate. Although the activity of pectinase varied depending on the nature of the carbon source used, namely glucose, sucrose, arabinose, and pectin, increasing the concentration of each of these sources in the medium promoted the pectinase activity of the selected strain. The highest activities were observed at the highest tested concentrations. These observations are consistent with those of Mat Jalil *et al.* (2023), who also reported improved pectinase enzyme activity with increasing the carbon source concentration. However, they differ from those reported by KC *et al.* (2020), who observed a decrease in pectinase production by *Aspergillus niger* when the substrate concentration increased. Similarly, Satpathy *et al.* (2025) showed that a moderate pectin concentration was conducive to pectinase production. On the contrary to the trend observed in the present study, where pectin activity increased up to the highest tested concentration, a previous study reported by Haile and Ayele (2022) indicated that excessive concentrations of pectin can lead to a decrease in pectin activity, probably due to substrate inhibition or catabolite repression. Given that the nitrogen source plays a crucial role in enzymatic activity, various nitrogen compounds were considered to study

their influence. The results showed a variation in the enzyme activity depending on the type of nitrogen compound. Among these compounds, $(NH_4)_2HPO_4$ proved to be the best source with an activity of 13.04 IU/ml (Figure 4c), and was followed by yeast extract. Peptone led to the lowest values. A considerable increase was observed as the concentration of the different compounds increased. The superiority of the observed inorganic nitrogen source suggested a rapid assimilation and an inducing effect on the metabolism, unlike the organic sources, which induced a repressive effect. Contrary to our results, Shet *et al.* (2022) reported higher activity with peptone, an organic nitrogen source. Furthermore, Satpathy *et al.* (2025) reported a negative impact of the inorganic nitrogen sources. However, El-Enshasy *et al.* (2018) reported that $(NH_4)_2HPO_4$ was the most influential nitrogen source on pectinase production. This difference could be due to the nature of the strain studied.

To determine the optimum pH for pectinase production, the bacterial culture was incubated at different pH levels ranging from 2 to 10. Variations in the pectinase activity were observed at the different tested pH levels. These variations were attributed to changes in the ionization state of the amino acids in the enzyme's active site, thus affecting its affinity for its substrate. As shown in Figure 4d, the maximum activity (14.29 IU/ml) was obtained at pH 7, followed by pH 6 (12.61 IU/ml). The enzyme retained more than 50% of its activity over a wide pH range from 4 to 8. The optimum pH obtained in this study is consistent with that reported for many other bacterial pectinases. However, a pH of 4.5 was reported by Alqahtani *et al.* (2022) as optimum for the pectinase of *B. siamensis* and a pH of 9 was recorded by Abdollahzadeh *et al.* (2020) on optimum for the pectinase produced by *Enterobacter* sp. MF84. The observed differences can be assigned to the genetic variability of the tested bacterium.

Optimization of pectinase production using response surface methodology

Based on the results of single-factor optimization design, pectin, $(NH_4)_2HPO_4$, and pH 7 were selected as optimum components of the culture medium. Data from 30 experiments obtained using a centred composite design were fitted to a second-order polynomial model with Y representing the response (Equation 5).

$$Y1 = 11.74 + 1.50X_1 - 0.44X_2 - 2.62X_3 + 0.31X_4 + 1.49X_1X_2 - 1.42X_1X_3 - 0.033X_1X_4 - 0.23X_2X_3 + 0.76X_2X_4 - 0.23X_3X_4 - 0.72X_1^2 - 1.38X_2^2 - 2.20X_3^2 - 0.12X_4^2 \quad ...(5)$$

Where; Y represents the total pectinase activity (UI/ml); X_1, X_2, X_3 and X_4 respectively represent the independent variables used for the optimization of the production medium, namely pectin (g/l), $(NH_4)_2HPO_4$ (g/l), temperature (°C), and K_2HPO_4 (g/l).

The influence of each factor and–its interactions on pectinase production by the bacterium was analyzed using RSM. According to the experimental response, the activity of pectinases produced by *B. amyloliquefaciens* PQ845326 ranged from 0.1214 to 19.3953 IU/ml (Table 4), demonstrating the influence of the different parameters and an optimization yield of 160 folds. The maximum pectinase activity was observed with pectin (10 g/l), $NH_4)_2HPO_4$ (3 g/l), a temperature of 32.5°C, and K_2HPO_4 (3 g/l), corresponding to series number 1. The maximum enzyme activity recorded in this study was remarkably higher than that obtained by Ahmed *et al.* (2021) in *Aspergillus niger*, where a maximum production of 0.6173 µmol/ml was recorded. Furthermore, an optimization rate of 9 was reported by Satpathy *et al.* (2024), which remained lower than that obtained in the present work, thus indicating a better optimization efficiency. However, superior performance was reported by El-Enshasy *et al.* (2018) for *Aspergillus niger* pectinase, with activities reaching 101.06 IU/ml in the most optimized medium. ANOVA of the model showed a p-value < 0.05, suggesting that the variables studied significantly influenced the response. Moreover, the linear terms X_1 and X_3 and the quadratic terms X^2_2 and X^2_3 were highly significant (p < 0.05), indicating a direct effect of the pectin concentration and the incubation temperature on pectin production. The significance of the quadratic terms X^2_2 and X^2_3 highlights a non-linear relationship between these factors and pectinase production, indicating the existence of optimal values for $(NH_4)_2HPO_4$ concentration and temperature. These values were determined from the response surface model, namely 3 g/l for $(NH_4)_2HPO_4$ and 32.5 °C. However, the interaction among the different variables was not substantial, indicating the independence between their effects. The considerable effect of pectin suggested that the carbon source played a crucial role in the bacterial pectinase production. These results are consistent with those of El-Enshasy *et al.* (2018), who reported

pectin's role as an inducer, potentially stimulating the expression of genes encoding for pectinase synthesis. Furthermore, the optimal concentration observed with the nitrogen source could be explained by its limiting effect at low concentrations and its inhibitory effect at high concentrations. On the contrary to the results observed in this study, Umar *et al*. (2023) did not observe a considerable quadratic effect associated with the nitrogen source. Their study instead showed a remarkable linear effect of this factor on pectinase production. The combined significance of the linear and the quadratic effects of temperature, with a negative quadratic coefficient, was typical to enzymatic systems and corroborates observations reported by Zhang *et al*. (2025) that temperature exerted a major control over the pectinase enzyme production. At low temperatures, pectinase production was limited by reduced substrate flux across the bacterial membrane. Conversely, above the optimal temperature, enzyme denaturation can occur, leading to reallocation of the energy resources towards growth at the expense of secondary metabolites production such as enzymes. These results are consistent with those reported by Afzia *et al*. (2024), who also showed that pectinase activity decreased above the optimal temperature.

The coefficient of determination (R^2) of the model was 0.7139, indicating that 71.39% of the observed variation in enzyme activity can be assigned to the studied factors. However, the adjusted R^2 was lower at 0.4469, indicating that the model's explanatory power decreased after accounting for the number of included terms. This difference between R^2 and adjusted R^2 suggested that some model terms may contribute little, or that experimental variability was not fully explained by the model. The predicted R^2 was 0.7862, suggesting an acceptable predictive power under the experimental conditions studied. Thus, the model can be considered useful for guiding the optimization of pectinase production, but its explanatory power remains only moderate. Furthermore, the goodness-of-fit test was not significant (Table 5). The non-significant lack of fit indicated that no statistically significant difference was observed between the experimental values and those predicted by the model, suggesting that the quadratic model remained acceptable for describing the response in the experimental domain studied. These results are broadly consistent with previous studies conducted on optimizing pectinase production using RSM (Sharif *et al*., 2023; Kottilingal *et al*., 2026).

Figure 5 shows 3D response surfaces with enzyme activity (U/ml) on the vertical axis representing the response variable. These three-dimensional response surfaces were generated to assess the combined effect of the two variables and identify the presence and the magnitude of their interactions on the enzyme activity, while the other variables were held constant at their fixed levels. Figure 5a illustrates the evolution of pectinase activity as a function of pectin and $(NH_4)_2HPO_4$ concentrations. The surface exhibited a parabolic shape with an optimum enzyme activity, which explained the remarkable quadratic effect of the factors X^2_1 and X^2_2. The pectinase activity increased with pectin concentration up to 10 g/l, then decreased. It was also increased with $(NH_4)_2HPO_4$ concentration up to an optimum of 3 g/l, then decreased. Thus, the most favourable conditions observed on this surface corresponded approximately to 10 g/l pectin and 3 g/l $(NH_4)_2HPO_4$. However, this apparent optimum range must be attributed to the individual and quadratic effects of the two factors.

Figure 5b shows the combined effect of pectin and temperature on pectinase activity. The response surface shows an upward slope in its initial region, suggesting an increase in activity as both pectin concentration and temperature increase. This trend can be explained by the inductive role of pectin in pectinase production. Similar observations have been reported for *Aspergillus niger* pectinase, where the addition of citrus pectin to the culture medium remarkably increased the pectinase activity (Mat Jalil *et al*., 2023). However, this increase was not linear with temperature (X_3). Above a certain temperature, the variation became moderate and slightly downward, indicating the presence of an optimum or a quadratic effect. Pectin acts as a factor promoting enzyme production, while temperature modulates this response. The contour lines were elliptical, confirming the presence of an optimum and a substantial interaction between the two independent factors and explaining why the effect of one factor depends on the level of the other. This type of interaction was also reported by Satpathy *et al*. (2025), where pectin and temperature were among the factors influencing *B. siamensis* pectinase. The maximum response was obtained with a high pectin concentration within the limits of the experimental range studied, and at a temperature of 32.50 °C. The combined effect of pectin and K_2HPO_4 is shown in Figure 5c. Unlike the previous figures, it exhibited a nearly flat shape, indicating a small

contribution from the quadratic terms X^2_2 and X^2_3, and therefore the absence of a marked optimum pectinase activity as a function of these two factors. The response varied very little when these two factors were modified simultaneously. The broad, slightly constricted contour lines without closed elliptical contours showed a negligible interaction between the two factors. These results suggested that pectin and K_2HPO_4 exerted a moderate effect on enzyme activity, without a marked combined influence. Figure 5d shows the three-dimensional response surface and its contour lines illustrating the combined effect of $(NH_4)_2HPO_4$ and temperature on pectinase activity. The curve was convex and slightly upward-sloping as temperature and $(NH_4)_2HPO_4$ concentration increased, reaching an optimal activity of 15 IU/ml. However, a slight slope was observed, indicating that the activity did not evolve in a strictly linear fashion. Rather, it followed a quadratic trend, suggesting the existence of a favourable range. To maximize the response, an $(NH_4)_2HPO_4$ concentration of 3 g/l and a temperature of 32.5°C should be selected. The contour lines were concentric, indicating irregular variation in pectinase activity and confirming the existence of a local optimum condition for pectinase production through the combination of the two factors. Furthermore, the oval shape of the contour lines and their slightly more elongated orientation along the X_2 axis indicated a weak interaction between the two factors and a more pronounced influence of temperature than $(NH_4)_2HPO_4$ on the response. This stronger influence of temperature can be explained by its direct impact on the microbial growth and cellular metabolism. In this regard, Kaur and Gupta (2017) showed that temperature is a key factor in pectinase production, while nitrogen sources play a more supportive nutritional role, the effect of which depends on the concentration used and the strain studied. Figure 5e shows the evolution of pectinase activity as a function of the concentrations of $(NH_4)_2HPO_4$ and K_2HPO_4, with the other factors held constant. The surface showed a progressive increase in activity up to an optimum, followed by a decrease beyond a certain concentration. This trend primarily reflected the individual and the quadratic effects of the two factors on pectinase production.

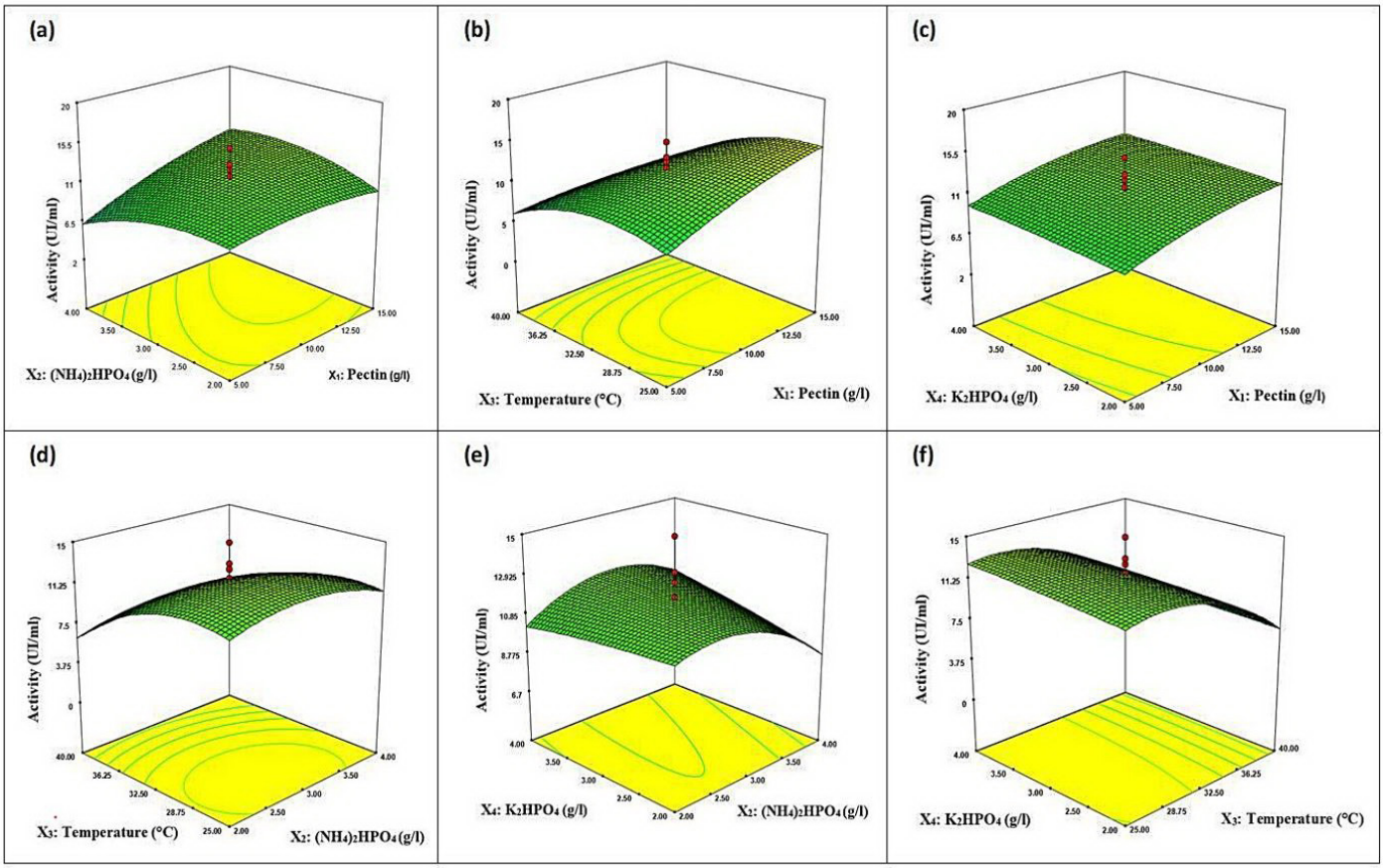

Figure 5: *Three-dimensional response surfaces illustrating the effect of culture factors on the pectinase production. (a) Effect of the combination pectin-$(NH_4)_2HPO_4$, (b) pectin- temperature, (c) pectin-K_2HPO_4, (d) $(NH_4)_2HPO_4$, temperature, (e) $(NH_4)_2HPO_4$- K_2HPO_4, and (f) temperature- K_2HPO_4. The two factors shown vary simultaneously, whereas the others are maintained at their central levels.*

The most favourable conditions observed for this response corresponded to a moderate combination of $(NH_4)_2HPO_4$ and K_2HPO_4. Despite the apparent joint variation observed on the response surface, the lack of significant interaction in the ANOVA suggested that the effects of $(NH_4)_2HPO_4$ and K_2HPO_4 were independent. On the contrary to the results of the present study, El-Enshasy *et al.* (2018) reported that the joint variation of the mineral nitrogen source and K_2HPO_4 influenced pectinase production. Figure 5f illustrates the variation of pectin activity as a function of K_2HPO_4 concentration and temperature, with pectin and $(NH_4)_2HPO_4$ concentrations held constant at their median values. The response surface showed an increase in enzyme activity up to a maximum, followed by a slight decrease. Increasing the temperature promoted the enzyme activity up to an optimum near 32.50 °C, beyond which the activity decreased slightly. Similarly, a moderate increase in K_2HPO_4 concentration appeared to improve the activity, whereas higher concentrations tended to be detrimental. The contour lines were nearly parallel, indicating a weak interaction between temperature and K_2HPO_4 concentration. This observation is consistent with the ANOVA results, which revealed no significant interaction between the factors. Thus, the observed variations in specific surface area appear to be mainly related to the individual and quadratic effects of the variables, rather than to a statistically significant synergy or antagonism between them. Among the variables studied, pectin appeared to be one of the most influential factors on the pectinase activity. This result is consistent with its role as an inducer of pectinase synthesis (Satpathy *et al.*, 2024). However, the beneficial influence of pectin appeared to be limited to a specific concentration range, with a maximum of 10 g/l, as indicated by the curvature of the response surface. Temperature was also an important factor in pectinase production. These results suggested that maximum pectinase activity was achieved at a moderate temperature near 32.5 °C. A lower temperature could limit the bacterial metabolic activity, while a higher temperature may affect the microbial growth, enzyme production, or enzyme stability (Ali *et al.*, 2025). Thus, optimizing pectinase production should consider the simultaneous adjustment of these two factors. In contrast, the other interactions among the factors were not statistically significant. Therefore, the effect of $(NH_4)_2HPO_4$ and K_2HPO_4 was primarily attributed to their own or quadratic effects within the experimental range studied, rather than to their interaction effects. $(NH_4)_2HPO_4$ appeared to promote the enzyme activity at intermediate concentrations, which could be explained by its role as a source of nitrogen and phosphorus, both of which were necessary for microbial metabolism (El-Enshasy *et al.* 2018; Lu *et al.*, 2025). This observation is consistent with the results of Joshi *et al.* (2006), who showed that supplementing the medium with 0.2% $(NH_4)_2HPO_4$ promoted the production of pectin esterase by *Aspergillus niger*. On the other hand, K_2HPO_4 exhibited a more moderate effect, indicating that it was not the primary factor governing the response. This trend aligns with the findings of Mohandas *et al.* (2018), who demonstrated that K_2HPO_4 had a weaker impact on pectinase production by the *B. sonorensis* strain. Thus, K_2HPO_4 appears to have contributed to the enzyme activity, but only secondarily, compared to the other factors such as pectin and temperature. Overall, maximum pectinase activity was observed under conditions combining a relatively high pectin concentration, intermediate concentrations of $(NH_4)_2HPO_4$ and K_2HPO_4, and a moderate temperature of approximately 32.5 °C. These results confirmed the importance of optimizing the nutritional and the physical parameters to improve the pectinase production. A recent work has reported that pectinase production by *Bacillus* strains was highly dependent on medium composition and incubation temperature, with optimal conditions varying with the bacterial strain and the substrate used (Kottilingal *et al.*, 2026). Thus, the optimal conditions for pectinase production by *B. velezensis*, as determined by this study, were 5.5 g/l pectin, 10 g/l yeast, 1 g/l glucose, pH 7, and 30 °C.

Conclusions and Recommendations

This study allowed the isolation, selection, and characterization of the pectinolytic bacteria from several decaying fruits. Among the isolates obtained, four showed high hydrolytic activity and were identified as: *Lactiplantibacillus plantarum*, *Acetobacter tropicalis*, *B. amyloliquefaciens*, and *B. subtilis*. Among these isolates, the *B. amyloliquefaciens*-related strain isolated from a decomposing cashew apple exhibited the highest pectinolytic index. Its γ-haemolytic profile indicated the absence of haemolysis under the tested conditions, supporting its selection for pectinase production optimization trials. Single-factor optimization revealed that pectin and $(NH_4)_2HPO_4$ were the best carbon and nitrogen

sources, respectively, for pectinase production, while pH 7 represented the optimum pH. Furthermore, multifactorial optimization using the centred composite design remarkably improved the production yield, increasing it 160-fold compared to the non-optimized condition. A maximum pectinase activity of 19.3953 IU/ml was obtained at 32.5 °C with 10 g/l of pectin, 3 g/l of $(NH_4)_2HPO_4$, and 3 g/l of K_2HPO_4, at pH 7. These obtained results suggested that the selected strain related to *Bacillus amyloliquefaciens* is a promising candidate for the production of pectinases used for biotechnological applications. In light of these results, further investigations are recommended to more precisely confirm the strain's taxonomic identification using deeper molecular methods. It is also recommended to further assess the selected strain safety, particularly before any application in the food industry. Furthermore, the enzyme produced should be purified and characterized to determine its biochemical properties, including thermal stability, pH stability, and behaviour in the presence of various ions or chemical agents. Moreover, it would also be relevant to evaluate the effectiveness of this pectinase in the various technological processes, such as clarifying or treating the cashew apple juice.

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Novelty Statement

This study proposes an optimized approach for pectinase production by *Bacillus amyloliquefaciens* isolated from a locally produced, decomposing food matrix, the cashew apple. It is distinguished by the combined study of four production factors: pectin, $(NH_4)_2HPO_4$, K_2HPO_4, and temperature. To our knowledge, few studies have simultaneously evaluated the combined effect of these four factors for optimizing pectinase production by *Bacillus* sp. The integration of a haemolysis assay further strengthened the assessment of this isolate's potential for food applications. The results obtained, with a maximum pectinase enzyme activity of 19.39 IU/ml under the optimized conditions, highlight the potential of this strain for pectinase production, particularly in the processing of cashew apple juice.

Author's Contribution

Tienbnoma Sandrine Ouedraogo, Boureima Kaboré and Iliassou Mogmenga: Conceptualization, methodology, investigation, software, writing original draft.
Mamounata Diao: Conceptualization, data curation, investigation, software, formal analysis, writing review and editing.
Fréderic Anderson Konkobo, Roger Dakuyo and Poussian Raymond Barry: Methodology, writing original draft.
Hemayoro Sama: data curation, formal analysis; writing original draft.
Samson Guenné, Crépin Ibingou Dibala and Kiessoun Konaté: Writing reviewing and editing.
Mamoudou Hama Dicko: Funding acquisition, supervision, validation.
The final version of the manuscript was approved by all the authors.

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Ethical approval
This study did not involve human participants, laboratory animals, or sensitive personal data. Therefore, ethical approval was not required.

Generative AI and AI assisted technology statement
The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interests
The authors have declared no conflicts of interest.

References

Aaisha, G. and Barate, D., 2016. Isolement et identification de bactéries pectinolytiques à partir d'échantillons de sol de la région d'Akola, en Inde. Int. J. Curr. Microbiol. App. Sci., 5(1): 514–521. https://doi.org/10.20546/ijcmas.2016.501.051

Abdel-Aziz, N.M., Atallah, A.G., Abdel-Aal,

S., Mohamed, E.A., Ibrahim, S.A. and Zein Elabedeen, A., 2019. Isolation, Identification and molecular characterization of pectinase producing bacterial isolate *Klebsiella oxytoca*. Arab Univ. J. Agric. Sci., 27(1): 553–561. https://doi.org/10.21608/ajs.2019.43665

Abdollahzadeh, R., Pazhang, M., Najavand, S., Fallahzadeh-Mamaghani, V. and Amani-Ghadim, A.R., 2020. Screening of pectinase-producing bacteria from farmlands and optimization of enzyme production from selected strain by RSM. Folia Microbiol., 65(4): 705–719. https://doi.org/10.1007/s12223-020-00776-7

Afzia, N., Shill, N., Kalita, B.J. and Nandan S., 2024. Optimization of conditions for production of pectinase in solid-state fermentation with *Aspergillus flavus* using dried Assam lemon peel powder as substrate, Measurement: Foods, 14: 100166. https://doi.org/10.1016/j.meafoo.2024.100166

Aguilar, G. and Huitron, C., 1987. Stimulation of the production of extracellular pectinolytic activities of *Aspergillus* sp. by galacturonic acid and glucose addition. Enzy. Microbial Technol., 9(11): 690-696. https://doi.org/10.1016/0141-0229(87)90129-3

Ahmed, T., Rana, M.R., Zzaman, W., Ara, R. and Aziz, M.G., 2021. Optimization of substrate composition for pectinase production from Satkara (*Citrus macroptera*) peel using *Aspergillus niger*-ATCC 1640 in solid-state fermentation. Heliyon, 7(10): e08133. https://doi.org/10.1016/j.heliyon.2021.e08133

Ali, I., Abdullah, R., Saqib, S., Nisar, K., Kaleem, A., Iqtedar, M., Iqbal, I. and Chen, X., 2025. Statistical optimization of pectinases from thermophilic *Aspergillus fumigatus* BT-4 employing response surface methodology through submerged fermentation using agricultural wastes. BMC Biotechnol., 25(1): 1–16. https://doi.org/10.1186/s12896-024-00942-6

Alqahtani, Y.S., More, S.S.R.K., Shaikh, I.A.K.J.A., More, V.S., Niyonzima, F.N., Muddapur, U.M. and 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

Amin, F., Haq, N.B. and Muhammad, B., 2019. Recent advances in the production strategies of microbial pectinases A review. Int. J. Biol. Macromol., 122: 1017-1026. https://doi.org/10.1016/j.ijbiomac.2018.09.048

Anand, G., Sangeeta, Y., Gupta, R. and Yadav, D., 2020. Pectinases: from microbes to industries. *Microorg.* Sustain. Environ. Health, 2020: 287-313. https://doi.org/10.1016/B978-0-12-819001-2.00014-0

Asmaa, B., Aicha, O., El-hadja, B.B. and Zaineb, H., 2020. Isolation and characterization of bacteria of the genera *Pseudomonas* and *Bacillus* with enzymatic potential from a volcanic soil of Ahagga. JSNEABA'20 (CP5.2).

Blanco Crivelli X., Cundon, C., Bonino, M.P., Sanin, M.S. and Bentancor, A., 2024. Le genre complexe et changeant *Bacillus*: une puissance bactérienne diversifiée pour de nombreuses applications. Bacteria, 3(3): 256-270. https://doi.org/10.3390/bacteria3030017

Cho, H.D., Kim, J.H., Won, Y.S., Moon, K.D. and Seo, K.I., 2019. Inhibitory effects of pectinase-treated prunus mume fruit concentrate on colorectal cancer proliferation and angiogenesis of endothelial cells. J. Food Sci., 84: 3284-3295. https://doi.org/10.1111/1750-3841.14824

Dashti, A., Jadaon, M., Abdulsamad, A. and Dashti, H., 2009. Heat treatment of bacteria: A simple method of DNA extraction for molecular techniques. Kuwait Med. J., 41(2): 117-122.

De Souza, T.S.P. and Kawaguti, H.Y., 2021. Cellulases, hemicellulases, and pectinases: Applications in the food and beverage industry. Food Bioprocess. Technol., 14: 1446–1477. https://doi.org/10.1007/s11947-021-02678-z

El-Enshasy, H.A., Elsayed, E.A., Suhaimi, N., Malek, R.A. and Esawy, M., 2018. Bioprocess optimization for pectinase production using *Aspergillus niger* in a submerged cultivation system. BMC Biotechnol., 18(1): 71. https://doi.org/10.1186/s12896-018-0481-7

Geris, R., Teles de Jesus, V.E., Ferreira da Silva, A. and Malta, M., 2024. Exploring culture media diversity to produce fungal secondary metabolites and cyborg cells. Chem. Biodiv., 21(3): e202302066. https://doi.org/10.1002/cbdv.202302066

Ghazala, I., Haddar, A., Romdhane, M. Ben and Ellouz-chaanouni, S., 2016. Screening and molecular identification of new microbial strains for production of enzymes of biotechnological

interest. Braz. Arch. Biol. Technol., 59(12): 1–12. https://doi.org/10.1590/1678-4324-2016150152

Govindaraji, P.K. and Vuppu, S., 2020. Characterisation of pectin and optimization of pectinase enzyme from novel *Streptomyces fumigatiscleroticus* VIT-SP4 for drug delivery and concrete crack-healing applications: An eco-friendly approach, Saudi J. Biol. Sci., 27(12): 3529-3540. https://doi.org/10.1016/j.sjbs.2020.07.024

Haile, M. and Kang, W.H., 2019. Isolation, Identification, and characterization of pectinolytic yeasts for starter culture in coffee fermentation. *Microorganisms*, 7(10):401. https://doi.org/10.3390/microorganisms7100401

Haile, S. and Ayele, A., 2022. Pectinase from microorganisms and its industrial applications. Sci. World J., 2022: 1881305. https://doi.org/10.1155/2022/1881305

Jalil, M.T.M. and Ibrahim, D., 2021. Partial purification and characterisation of pectinase produced by *Aspergillus niger* LFP-1 grown on pomelo peels as a substrate. Trop. Life Sci. Res., 32(1): 1–22. https://doi.org/10.21315/tlsr2021.32.1.1

Janda, J.M. and Abbott, S.L., 2007. *16S rRNA* gene sequencing for bacterial identification in the diagnostic laboratory: Pluses, perils, and pitfalls. J. Clin. Microbiol., 45(9): 2761-2764. https://doi.org/10.1128/JCM.01228-07

Jayani, R.S., Saxena, S. and Gupta, R., 2005. Microbial pectinolytic enzymes: A review. Process. Biochem., 40(9): 2931-2944. https://doi.org/10.1016/j.procbio.2005.03.026

Jin, L., Chao, P., Aihua, M., Mingqi, Z. and Zhong, H., 2024. An overview of microbial enzymatic approaches for pectin degradation. Int. J. Biol. Macromol., 254: 127804. https://doi.org/10.1016/j.ijbiomac.2023.127804

Joshi, V., Parmar, M. and Rana, N., 2006. Pectin esterase production from apple pomace in solid-state and submerged fermentations. Food Technol. Biotechnol., 44(2): 253-256.

Kamalambigeswari, R., Yadav, S., Sivaswamy, N. and Ushani, U., 2018. Isolation, identification, screening and optimization of pectinase producing soil fungi (*Aspergillus niger*). Int. J. Res. Pharm. Sci., 9: 762-768.

Kaur, S., Kaur, H.P., Prasad, T.B. and Bharti, E., 2016. Production and optimization of pectinase by *Bacillus* sp. isolated from vegetable waste soil. Indo Am. J. Pharm. Res., 6(1): 4185–4190.

Kaur, S.J. and Gupta, V.K., 2017. Production of pectinolytic enzymes pectinase and pectin lyase by *Bacillus subtilis* SAV-21 in solid state fermentation. Ann. Microbiol., 67: 333–342. https://doi.org/10.1007/s13213-017-1264-4

Kavitha, M., Raja, M. and Perumal, P., 2018. Evaluation of probiotic potential of *Bacillus* spp. isolated from the digestive tract of freshwater fish *Labeo calbasu* (Hamilton, 1822). Aquacult. Rep., 11(7): 59–69. https://doi.org/10.1016/j.aqrep.2018.07.001

Kc, S., Upadhyaya, J., Joshi, D.R., Lekhak, B., Kumar, C.D., Raj Pant, B., Raj Bajgai, T., Dhital, R., Khanal, S., Koirala, N. and 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

Kh, A.E.A., Attallah, A.G., Abdel-Aziz, N.M. and Khalil, B.E., 2022. Isolation, screening, and molecular identification of pectinase producers from fruits, vegetables, and soil samples. Egypt. Pharm. J., 21(3): 302–311. https://doi.org/10.4103/epj.epj_39_22

Khan, G., Fatima, S.T., Masood, R. and Umar, A., 2025. Economic-related aspects of pectinase in the textile industry. In: (ed. M. Arshad) enzymes in textile processing: A climate changes mitigation approach. SDGs and Textiles. Springer. https://doi.org/10.1007/978-981-97-8058-7_7

Kottilingal, L.S., Paramasivam, S., Sivaprakasam, S., Meera, C.R. and Chinnaiyan, U., 2026. Optimization of pectinase production from *Bacillus velezensis* isolated from agro-waste using response surface methodology. J. Pure Appl. Microbiol., 20(1): 459-469. https://doi.org/10.22207/JPAM.20.1.33

Kučuk, N., Primožič, M., Kotnik, P., Knez, Ž. and Leitgeb, M., 2024. Mango peels as an industrial by-product: A sustainable source of compounds with antioxidant, enzymatic, and antimicrobial activity. Foods, 13(4): 553. https://doi.org/10.3390/foods13040553

Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K., 2018. Mega X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol. Biol. Evol., 35: 1547–1549. https://doi.org/10.1093/molbev/msy096

Lee, Y., Kang, J., Hwang, J., Shin, D. M., Han, S. G. and Yoon, Y., 2023. Selection of *Debaryomyces hansenii* isolates to improve quality of dry fermented sausage. Int. J. Food Prop., 26(1): 2433–2454. https://doi.org/10.1080/10942912.2023.2244690

Lu, F., Tan, C., Li, H. and Qian, F., 2025. Optimization of pectinase production from silkworm excrement using *Aspergillus niger*. Fermentation, 11(6): 333. https://doi.org/10.3390/fermentation11060333

Mat Jalil, M.T., Zakaria, N.A., Salikin, N.H. and Ibrahim, D., 2023. Assessment of cultivation parameters influencing pectinase production by *Aspergillus niger* LFP-1 in submerged fermentation. J. Genet. Eng. Biotechnol., 21(1): 45. https://doi.org/10.1186/s43141-023-00510-z

Mercimek Takcı, H.A. and Turkmen, F. U., 2016. Extracellular Pectinase Production and Purification from a Newly Isolated *Bacillus subtilis* Strain. Int. J. Food Prop., 19(11): 2443–2450. https://doi.org/10.1080/10942912.2015.1123270

Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. J. Anal. Chem., 31: 426-428. https://doi.org/10.1021/ac60147a030

Mohammed, M.R., Ajiboye, A.E. and Abdulmumini, S., 2021. Activité pectinolytique d'*Aspergillus Niger* et d'*Aspergillus flavus* cultivés sur une peau de pamplemousse (*citrus Parasidis*) en fermentation à l'état solide. J. Pure Appl. Sci., 27(2): 93–105. https://doi.org/10.4314/gjpas.v27i2.2

Mohandas, A., Sindhu, R., Binod, P., Abraham, A., Mathew A.K. and Pandey A., 2018. Production of Pectinase from *Bacillus sonorensis* MPTD1. Food Technol. Biotechnol., 56(1): 110-116. https://doi.org/10.17113/ftb.56.01.18.5477

Mulluye, K. and Atnafu, Y., 2022. Application biotechnologique de la pectinase. J. Pharm. Sci. Res., 13(8): 3069-3077.

Murad, H.A. and Azzaz, H.H., 2011. Microbial pectinases and ruminant nutrition. Res. J. Microbiol., 6: 246-269. https://doi.org/10.3923/jm.2011.246.269

Nwagu, T.N., Ugwuodo, C.J., Onwosi, C.O. Inyima, O., Uchendu O. C. and Akpuru, C., 2020. Evaluation of the probiotic attributes of *Bacillus* strains isolated from traditional fermented African locust bean seeds (*Parkia biglobosa*), "daddawa". Ann. Microbiol., 70: 20. https://doi.org/10.1186/s13213-020-01564-x

Okonji, R.E., Itakorode, B.O., Ovumedia, J.O. and Adedeji, O.S., 2019. Purification and biochemical characterization of pectinase produced by *Aspergillus fumigatus* isolated from soil of decomposing plant materials. J. Appl. Biol. Biotechnol., 7(3): 1–8. https://doi.org/10.7324/JABB.2019.70301

Oumer, O.J. and Abate, D., 2017. Characterization of Pectinase from *Bacillus subtilis* Strain Btk 27 and its potential application in removal of mucilage from coffee beans. Enzyme Res., 2017: 7686904. https://doi.org/10.1155/2017/7686904

Oumer, O.J. and Abate, D., 2018. Screening and molecular identification of pectinase producing microbes from coffee pulp. BioMed. Res. Int., 2018(1): 2961767. https://doi.org/10.1155/2018/2961767

Owusu, S.B., Zakpaa, H.D., Borigu, M., Duwiejuah, A.B., Boakye, S.O., Zakpaa, H.D. and Borigu, M., 2024. Isolation, screening and identification of pectinolytic fungi from the soil of decomposed plant materials Preprints, pp. 0–11. https://doi.org/10.20944/preprints202412.0018.v1

Panchal, H., Shaikh, M., Yadav, N., Tadvi, D. and Choudhary, S.T., 2026. Isolation, screening, and optimization of CMCase production from *Bacillus pumilus* A5 isolated from the fecal sample of Bos indicus. J. Appl. Biol. Biotechnol., 14(3): 165-172. https://doi.org/10.7324/JABB.2026.289138

Priest, F.G., 1977. Extracellular enzyme synthesis in the genus *Bacillus*. Bacteriol. Rev., 41(3): 711–753. https://doi.org/10.1128/br.41.3.711-753.1977

Priyanka, S.B. and Prajna M.U., 2019. Isolation, Purification and Characterization of Pectinase enzyme from *Streptomyces* ther *mocar boxydus*. J. Clin. Microbiol. Biochem. Technol., 5: 1–6. https://doi.org/10.17352/jcmbt.000031

Qureshi, Z.A., Paterson, D.L., Potoski, B.A., Kilayko, M.C., Sandovsky, G., Sordillo, E. and Doi, Y., 2012. Résultats du traitement de la bactériémie à *Klebsiella pneumoniae* productrice de KPC: Supériorité des schémas antimicrobiens combinés. Antimicrob. Agents Chemother., 56(4): 2108-2113. https://doi.

org/10.1128/AAC.06268-11

Ramin, K.I. and Allison, S.D., 2019. Bacterial tradeoffs in growth rate and extracellular enzymes. Front. Microbiol., 20(10): 2956. https://doi.org/10.3389/fmicb.2019.02956

Roosdiana, A., Prasetyawan, S., Mahdi, C. and Sutrisno, S., 2013. Production and characterization of *Bacillus firmus* pectinase. J. Pure Appl. Chem. Res., 2(1): 35–41. https://doi.org/10.21776/ub.jpacr.2013.002.01.111

Ruchika, A., Tanmay, D. and Javed, S., 2020. Extraction of pectinase from Candida isolated from textile mill effluent and its application in bio-scouring of cotton. Sustain. Chem. Pharm., 17: 100291. https://doi.org/10.1016/j.scp.2020.100291

Samanta, S., 2019. Microbial pectinases: A review on molecular and biotechnological perspectives. J. Microbiol. Biotech. Food Sci., 9: 248-266. https://doi.org/10.15414/jmbfs.2019.9.2.248-266

Sanger, F., Nicklen, S. and Coulson, A.R., 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. U.S.A., 74(12): 5463-5467. https://doi.org/10.1073/pnas.74.12.5463

Satpathy, A., Mukherjee, K. and Nigam, V.K., 2024. Batch cultivation and optimization of pectinase production using *Bacillus* sp. (BIOSMNF02) through RSM-D-optimal quadratic model. Biomass Conv. Bioref., 14: 31979–31990. https://doi.org/10.1007/s13399-023-04915-1

Satpathy, A., Mukherjee, K. and Nigam, V.K., 2025. Improved production of thermostable pectinase from novel *Bacillus siamensis* (BIOSMNF45) using computational-conventional approach. Sci. Rep., 15: 11489. https://doi.org/10.1038/s41598-024-77167-2

Shaik, L. and Chakraborty, S., 2022. Effect of pH and total fluence on microbial and enzyme inactivation in sweet lime (*Citrus limetta*) juice during pulsed light treatment. J. Food Process. Preserv., 46: e16749. https://doi.org/10.1111/jfpp.16749

Sharif, S., Shah, A.H., Fariq, A., Jannat, S., Rasheed, S. and Yasmin, A., 2023. Optimization of amylase production using response surface methodology from newly isolated thermophilic bacteria, Heliyon, 9(1): e12901. https://doi.org/10.1016/j.heliyon.2023.e12901

Sharma, H.P., Patel, H. and Sugandha., 2017. Enzymatic added extraction and clarification of fruit juices–A review. Crit. Rev. Food Sci. Nutr., 57(6): 1215–1227. https://doi.org/10.1080/10408398.2014.977434

Sheladiya, P., Kapadia, C., Prajapati, V. El-Enshasy, H.A., Malek, R.A., Marraiki, N., Zaghloul N.S.S. and Sayyed., R.Z., 2022. Production, statistical optimization, and functional characterization of alkali stable pectate lyase of *Paenibacillus lactis* PKC5 for use in juice clarification. Sci. Rep.,12: 7564. https://doi.org/10.1038/s41598-022-11022-0

Shet, A.R., Muhsinah, A. Bin, Alsayari, A., Achappa, S., Desai, S.V., Mahnashi, M.H., Muddapur, U.M., Shaikh, I.A., Mannasaheb, B.A. and Khan, A.A., 2022. Media optimization by response surface methodology for the enhanced production of acidic extracellular pectinase by the indigenously isolated novel strain *Aspergillus cervinus* ARS2 using solid-state. Fermentation, 8(10). https://doi.org/10.3390/fermentation8100485

Shrestha, S., Khatiwada, J.R., Zhang, X., Chio, C., Laurel, A., Kognou, M., Chen, F., Han, S., Chen, X. and Qin, W., 2021. Screening and molecular identification of novel pectinolytic *Bacteria* from forest soil. Fermentation, 7(40): 1–11. https://doi.org/10.3390/fermentation7010040

Sorokulova, I.B., Pinchuk, I.V., Denayrolles, M., Osipova, I.G., Huang, J.M., Cutting, S.M. and Urdaci, M.C., 2008. The safety of two *Bacillus* probiotic strains for human use. Dig. Dis. Sci., 53(4): 954-963. https://doi.org/10.1007/s10620-007-9959-1

Sui, X., Guo, L., Bao, Z., Xian, M., Zhao, G., 2025. Efflux pumps and porins enhance bacterial tolerance to phenolic compounds by inhibiting hydroxyl radical generation. microorganisms, 13: 202. https://doi.org/10.3390/microorganisms13010202

Sukmawati, D., Adisyahputra, Hasanah, R., Al-Husna, S.N., Ratnaningtyas N.I., Aksoy, A. and El-Enshasy, H.A., 2025. Optimization of carbon and nitrogen source on the production of pectinase enzymes in solid-state fermentation by *Cyberlindnera fabianii* UNJCC Y-144 and *Candida tropicalis* UNJCC Y-148-149 Isolated from Lai Fruit. J. Pure. Appl. Microbiol., 19(2): 1010-1026. https://doi.org/10.22207/JPAM.19.2.04

Thakur, P., Singh, A.K. and Mukherjee, G., 2021.

Isolation and characterization of alkaline pectinase productive *Bacillus tropicus* from fruit and vegetable waste dump soil. Braz. Arch. Biol. Technol., 64: 1–14. https://doi.org/10.1590/1678-4324-2021200319

Umar, M., Khan, A.R., Hayat, I., Hayat, P., Rehman, A., Mujaddad Ur, S., Tawaf, A.D., Turki M., Hadrach, S. and Mohammed, B., 2023. Screening and optimization of extracellular pectinase produced by *Bacillus thuringiensis* SH7. Open Chem., 21(1): 20220358. https://doi.org/10.1515/chem-2022-0358

Yoon, S.H., Ha, S.M., Kwon, S., Lim, J., Kim, Y., Seo, H. and Chun, J., 2017. Introducing EzBioCloud: A taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int. J. Syst. Evol. Microbiol., 67(5): 1613–1617. https://doi.org/10.1099/ijsem.0.001755

Zhang, Q.H., Ye, M., Wu, X.Y., Ren SX, Zhao, M., Zhao, C.J., Fu, G., Shen, Y., Fan, H.Y., Lu, G., Zhong, M., Xu, XR., Han, Z.G., Zhang, J.W., Tao, J., Huang, Q.H., Zhou, J., Hu, G.X., Gu, J., Chen, S.J. and Chen, Z., 2000. Cloning and functional analysis of cDNAs with open reading frames for 300 previously undefined genes expressed in CD34+ hematopoietic stem/progenitor cells. Genome Res., 10(10): 1546-1560. https://doi.org/10.1101/gr.140200

Zhang, S.D., Chen, L.L., Li, C.Y., Long, X.Q., Yang, X., He, X.D., Du, L.W., Yang, H.F. and Ling, L.Z., 2025. Optimization of cultural conditions for pectinase production by Diaporthe isolate Z1-1N and its pathogenicity on kiwifruit. PeerJ., 13: e19207. https://doi.org/10.7717/peerj.19207