Isolation And Production of Thermostable Pectinase Enzyme from Rajwadi Hot Water Spring, Maharashtra

Sonali Shrikant Patil[1], Kavathe Shubham Anant[2]
[1,2]*Department of Microbiology, Rajarshi Shahu Mahavidyalaya, Latur (Empowered Autonomous Institution), Maharashtra, India*

***Abstract*—Hot water springs are geochemically distinctive environments that sustain thermophilic microbial communities of considerable biotechnological relevance. The Rajwadi hot water spring, part of the Tural–Rajwadi geothermal system in Ratnagiri district, Maharashtra, India, maintains water temperatures between 50°C and 61°C and represents a poorly explored reservoir of thermostable enzyme-producing microorganisms. The present study was undertaken to isolate thermophilic bacteria from water and sediment samples collected at Rajwadi, screen them for pectinase production, optimize fermentation conditions, and assess the thermal stability of the crude enzyme extract. Enrichment in Nutrient Broth at 55°C yielded thermophilic colonies on Pectin Agar, from which a promising pectinase-producing isolate was selected on the basis of halo zone formation upon iodine flooding. Gram staining identified the isolate as a Gram-positive, short rod-shaped bacterium with colony characteristics — circular form, off-white colour, smooth surface, convex elevation, and mucoid consistency — consistent with Bacillus-like thermophiles documented from Indian geothermal sites [14,18]. Pectinase production was carried out in Pectin Broth at 55°C, over 72–96 hours, and the crude enzyme was harvested by centrifugation at 10,000 rpm. Enzyme activity, confirmed by the disc diffusion plate assay, demonstrated clear zones of pectin hydrolysis at 55°C, validating thermostable pectinase activity. These results establish the Rajwadi hot spring as a viable source of thermostable biocatalysts with potential applications in fruit juice clarification, textile retting, paper and pulp processing, and food biotechnology.**

***Index Terms*—thermostable pectinase; thermophilic bacteria; Rajwadi hot spring; geothermal; pectin degradation; Bacillus; Maharashtra; enzyme production; disc diffusion**

I. INTRODUCTION

1.1. Pectinases: Definition, Classification, and Industrial Significance

Enzymes are macromolecular biological catalysts that accelerate biochemical transformations with high specificity and efficiency under mild conditions. Among industrially important enzyme classes, pectinases occupy a prominent position owing to their ability to degrade pectin — a complex heteropolysaccharide that forms a major structural component of the primary cell walls and middle lamellae of higher plants [11,13]. Pectin consists principally of a linear backbone of α-1,4-linked D-galacturonic acid residues — homogalacturonan — partially methyl-esterified at the C-6 carboxyl groups, with branched hairy regions containing rhamnogalacturonan I and II substituted with arabinose, galactose, and other neutral sugars [9,11].

Pectinases are broadly classified into four groups: (i) pectin methylesterase (PME), which removes methyl groups from high-methoxyl pectin to yield low-methoxyl pectin; (ii) polygalacturonases (PG), which hydrolyse α-1,4-glycosidic bonds between galacturonic acid residues through endo- and exo-acting mechanisms; (iii) pectate lyases (PL), which cleave de-methylated galacturonans via β-elimination to produce unsaturated oligogalacturonates; and (iv) pectin lyases (PNL), which perform the same β-elimination directly on methyl-esterified pectin [12,13]. Together, these enzymes bring about depolymerisation and solubilisation of pectin, finding use across several industries — notably in fruit juice clarification and viscosity reduction, textile fibre retting, paper and pulp processing, coffee and cocoa fermentation, and bioethanol production from pectin-rich agricultural residues [12,13].

1.2. Thermostable Enzymes and the Case for Extremophile Sources

Conventional mesophilic pectinases, typically sourced from *Aspergillus niger* and related fungi, lose appreciable activity above 45–50°C, which restricts their use in high-temperature industrial operations such as hot-break juice processing and pressurised steam treatments [18,19]. This constraint has directed considerable research interest toward thermophilic microorganisms — broadly defined as organisms with optimal growth between 45°C and 80°C, with moderate thermophiles thriving between 45°C and 60°C — as sources of enzymes that remain stable and active at elevated temperatures. The structural basis of this thermostability lies in tighter protein folding, a greater density of hydrogen bonds and salt bridges, stronger hydrophobic core interactions, and more extensive disulphide bonding compared with their mesophilic counterparts [19]. These features collectively resist unfolding at temperatures that would readily denature equivalent mesophilic proteins.

Natural geothermal hot springs rank among the richest environments for isolating thermophilic and hyperthermophilic microorganisms. Their sustained elevated temperatures, distinctive mineral profiles, and varied pH regimes impose selective pressures that have shaped specialised enzymatic systems over geological timescales [18]. In India, hot spring systems along the western coastal geothermal belt — particularly in the Konkan region of Maharashtra — have received comparatively limited microbiological attention, despite the promise they hold for industrial enzyme discovery [1,14]. Saranraj and Naidu [3] observed that surveys of Indian hot springs consistently reveal Bacillus-dominated communities with broad enzymatic versatility; yet enzyme characterisation from these sites remains incomplete relative to the more extensively studied Himalayan and Tibetan geothermal systems.

1.3. Rajwadi Hot Water Spring: Geothermal Context

The Rajwadi hot water spring is a natural geothermal feature located near Sangameshwar, Ratnagiri district, Maharashtra, India, forming part of the Tural–Rajwadi geothermal system along the western coastal geothermal belt. The spring is underlain by Deccan Trap basalt formations through which groundwater, heated by crustal geothermal gradients of approximately 260°C/km, returns to the surface via hydrothermal convection through rock fractures. Water temperatures at the site range between 50°C and 61°C throughout the year, with the water notably enriched in dissolved hydrogen sulphide and carbon dioxide. The site encompasses approximately 14 individual hot water pools, some developed as bathing tanks, and carries cultural significance as a sacred site visited during festivals such as Mahashivratri. These physico-chemical conditions — sustained high temperature, sulphur-rich chemistry, and circum-neutral to slightly acidic pH — collectively create a selective thermal environment well suited to the enrichment of thermophilic microorganisms with industrial enzyme potential.

1.4. Mechanism of Pectin Degradation by Thermostable Pectinase

The enzymatic breakdown of pectin by thermostable pectinases generally proceeds in two coordinated phases: de-esterification followed by depolymerisation. In the first phase, pectin methylesterase removes methyl ester groups from high-methoxyl pectin, releasing methanol and converting the substrate to low-methoxyl pectin with exposed carboxyl groups accessible to hydrolytic and eliminative enzymes [11,13]. In the second phase, polygalacturonases cleave the α-1,4-glycosidic bonds between galacturonic acid residues — either randomly through endo-cleavage or processivity from the terminal end through exo-cleavage — generating oligogalacturonate fragments. Concurrently, pectate lyases act on de-methylated pectin through β-elimination to yield unsaturated oligogalacturonates bearing a Δ-4,5 double bond at the non-reducing terminus, while pectin lyases carry out the equivalent reaction directly on methylated pectin [13]. At the elevated temperatures characteristic of thermostable pectinase activity (55–80°C), substrate viscosity decreases and molecular diffusion rates increase, accelerating the overall rate of pectin solubilisation and making such enzymes well suited to continuous-flow industrial processes [18,19].

1.5. Scope of the Present Investigation

The Rajwadi spring system, despite its well-documented geothermal character, has not previously been examined as a source of pectinase-producing thermophiles. The present investigation was therefore

designed to isolate and characterise such organisms from water and sediment samples collected at the site, screen isolates for pectinase activity using the iodine halo assay, produce crude enzyme through fermentation in pectin broth, and confirm thermostable activity by disc diffusion assay at 55°C. This work adds to the growing body of evidence that Indian geothermal springs represent underexplored but promising reservoirs of industrially relevant biocatalysts

II. MATERIALS AND METHODS

2.1. Study Site and Sample Collection

Water samples were collected from the Rajwadi hot spring, located near Sangameshwar, Ratnagiri district, Maharashtra, India, approximately 375 km south of Mumbai. The spring forms part of the Tural–Rajwadi geothermal system along the western coastal geothermal belt. Water temperature at the collection point was recorded as 50–61°C; on-site pH measurement using wide-range pH paper indicated a mildly acidic reaction, consistent with the CO_2- and sulphur-rich chemistry of the spring water. Hot water (500 mL) was collected aseptically in pre-sterilised, wide-mouth thermos flasks to minimise temperature loss during transport. Samples were transferred to the laboratory under insulated conditions and processed within six hours of collection.

2.2. Culture Media

Two purpose-formulated media were prepared for this study:

Table 1. Composition of Pectin Agar isolation and screening medium (per 100 mL, pH 5.0).

Sr. No.	Component	Amount
1	Pectin	1 g
2	Yeast Extract	0.5 g
3	$(NH_4)_2SO_4$	0.2 g
4	KH_2PO_4	0.1 g
5	$MgSO_4 \cdot 7H_2O$	0.05 g
6	NaCl	0.05 g
7	Agar	1.5 g
8	Distilled water	100 mL
9	pH	5.0

Table 2. Composition of Pectin Broth production medium (per 100 mL, pH 5.0).

Sr. No.	Component	Amount
1	Pectin	1 g
2	Yeast Extract	0.5 g
3	$(NH_4)_2SO_4$	0.2 g
4	KH_2PO_4	0.1 g
5	$MgSO_4 \cdot 7H_2O$	0.05 g
6	NaCl	0.05 g
7	Distilled water	100 mL
8	pH	5.0

Both media were sterilised by autoclaving at 121°C, 15 psi, for 15 minutes. pH was adjusted to 5.0 prior to autoclaving using dilute HCl or NaOH as required. Nutrient Agar and Nutrient Broth were additionally prepared for enrichment and inoculum development, respectively.

2.3. Enrichment and Isolation of Thermophilic Microorganisms

Five millilitres of the collected hot spring water was added to 100 mL of Nutrient Broth in a 250 mL Erlenmeyer flask and incubated at 55°C for 7 days without shaking to enrich for thermophilic heterotrophs. Development of visible turbidity confirmed active microbial growth. Aliquots of the enriched culture were diluted serially in sterile phosphate buffer saline (0.1 M, pH 7.0) and plated on Pectin Agar by the spread plate method. Plates were incubated at 55°C for 24–48 hours. Morphologically distinct colonies were sub-cultured on Nutrient Agar slants and maintained at 4°C for further study.

2.4. Screening for Pectinase Production — Iodine Halo Assay

Pure cultures from Nutrient Agar slants were spot-inoculated onto Pectin Agar plates and incubated at 55°C for 24–48 hours. After sufficient colony development, plates were flooded with Gram's iodine solution, which was discarded after 1–2 minutes. Iodine reacts with residual pectin in the agar to produce a dark brown coloration; clear zones surrounding colonies indicate extracellular pectinase has hydrolysed the pectin and rendered it unresponsive to the iodine reaction. Halo diameter served as a qualitative indicator of pectinase productivity, and the isolate displaying the widest and most clearly defined halo was selected for further characterisation [11,14].

2.5. Morphological Characterisation and Gram Staining

Colony characteristics of the selected isolate — size, shape, colour, margin, surface texture, elevation, opacity, and consistency — were recorded after 48 hours of growth on Pectin Agar at 55°C. Gram staining was performed on heat-fixed smears from 24-hour Nutrient Agar cultures using the standard four-step protocol: crystal violet primary stain (1 min), Gram's iodine mordant (1 min), acetone–ethanol decolorisation (20 sec), and safranin counterstain (1 min). Smears were examined under oil-immersion at 100× magnification for cell shape, arrangement, and Gram reaction.

2.6. Pectinase Production — Submerged Fermentation

A 10 mL inoculum was prepared by transferring a loopful of the selected isolate from a Nutrient Agar slant into 10 mL of Nutrient Broth and incubating at 55°C for 24–48 hours with intermittent agitation until visible turbidity confirmed active growth. The inoculum was then transferred aseptically under laminar airflow into a 250 mL Erlenmeyer flask containing 200 mL of freshly prepared and sterilised Pectin Broth (pH 5.0). The production flask was sealed with non-absorbent cotton plugs and aluminium foil and incubated at 55°C for 72–96 hours on a rotary shaker at 120 rpm to ensure adequate aeration and nutrient mixing. Visible growth was assessed daily by inspecting for turbidity, flocculation, and broth colour change. Pectin served as the sole carbon source in the production medium, thereby imposing metabolic selection for pectinase-producing cells and encouraging enzyme induction [2,14].

2.7. Harvesting of Crude Pectinase

After completion of the production incubation period, the entire volume of production broth was centrifuged at 10,000 rpm for 15 minutes at 4°C to separate cell biomass from the enzyme-containing supernatant, with low-temperature centrifugation employed to minimise thermal stress on the enzyme during processing. The cell pellet was discarded and the supernatant was carefully decanted into a clean, sterile flask. A second centrifugation step at 10,000 rpm for 10 minutes at 4°C was performed to remove residual cellular debris and precipitates. The clarified supernatant, containing the extracellular crude pectinase, was collected and stored at 4°C for activity testing [16,17].

2.8. Enzyme Activity — Disc Diffusion Plate Assay

Pectinase activity of the crude enzyme extract was assessed using a disc diffusion method adapted from Kapoor et al. [14]. Whatman No. 1 filter paper discs (6 mm diameter) were sterilised by autoclaving and saturated with the crude enzyme extract (50 µL per disc). Discs were placed centrally on freshly solidified Pectin Agar plates and allowed to stand at room temperature for 20 minutes so that the enzyme could be absorbed into the surrounding agar before incubation commenced. Plates were then incubated at 55°C for 24 hours, after which they were flooded with Gram's iodine and observed for clear zones of pectin hydrolysis around the discs. The diameter of the clearance zone, measured from the outer edge of the disc to the boundary of the halo, was recorded as the direct indicator of pectinase activity and thermostability.

III. RESULTS AND DISCUSSION

3.1. Enrichment and Isolation on Pectin Agar

The enrichment culture in Nutrient Broth at 55°C yielded visible turbidity within 48 hours, confirming the presence of viable thermophilic microorganisms in the Rajwadi spring water. This relatively rapid onset of growth at 55°C reflects the pre-adaptation of the indigenous microbial community to the spring's sustained temperature range and is consistent with findings from comparable Indian geothermal sites [18]. Plating of diluted enrichment broth on Pectin Agar at 55°C produced multiple morphologically distinct colony types within 24–48 hours, ranging from small translucent pin-point colonies to larger, raised, off-white forms. Such diversity in colony morphology is well documented from Indian hot spring studies, where different thermophilic taxa occupy distinct ecological niches shaped by temperature gradients, mineral availability, and organic substrate concentrations [3,14].

3.2. Colony Characteristics and Gram Staining

The selected pectinase-producing isolate produced colonies with consistent morphology across repeated sub-cultures on Pectin Agar at 55°C. Colony characteristics are summarised in Table 3. The mucoid

consistency and convex elevation are of particular note in the context of thermophilic adaptation, as mucoid phenotypes in Bacillus-like thermophiles are frequently associated with exopolysaccharide production that contributes to structural stability at elevated temperatures [3,14].

Table 3. Colony characteristics of the pectinase-producing isolate on Pectin Agar at 55°C.

Sr. No.	Characteristics	Observation
1	Size	1 mm
2	Shape	Circular
3	Colour	Off-white
4	Margin	Entire
5	Surface	Smooth
6	Elevation	Convex
7	Opacity	Opaque
8	Consistency	Mucoid
9	Gram Nature	Positive

Gram staining of 24-hour cultures on Nutrient Agar revealed Gram-positive, short rod-shaped cells. This morphology is consistent with the genus *Bacillus*, which is widely reported as the dominant thermophilic pectinase producer from hot spring environments across India [3,11,14,18]. Kapoor et al. [14] similarly recovered a Gram-positive *Bacillus* sp. as the primary pectinase producer from Indian soil, while Saranraj and Naidu [3] identified *Bacillus stearothermophilus* and *B. licheniformis* as dominant pectinolytic thermophiles from Indian hot springs. Definitive species-level identification will require 16S rRNA gene sequencing, which is planned as the next stage of this work.

3.3. Screening for Pectinase Activity — Iodine Halo Assay

When the selected isolate was grown on Pectin Agar at 55°C for 48 hours and the plates subsequently flooded with Gram's iodine, distinct and well-defined zones of clearance were visible surrounding the colonies. The background agar retained the characteristic dark brown coloration of the iodine–pectin complex, while areas where extracellular pectinase had diffused into the medium appeared as clear, translucent haloes — directly confirming pectinase secretion and hydrolytic activity at the incubation temperature. The iodine halo assay, though qualitative, is a well-established and reliable primary screening tool for pectinase-producing microorganisms from environmental samples [11,13]. Zone formation at 55°C is itself evidence of thermostable enzyme activity, since mesophilic pectinases would not be expected to function effectively at this temperature.

3.4. Pectinase Production — Submerged Fermentation in Pectin Broth

Following inoculation into 200 mL Pectin Broth (pH 5.0) and incubation at 55°C for 72–96 hours on a rotary shaker at 120 rpm, visible growth was confirmed by progressive turbidity development and a gradual colour change in the broth to a pale golden-yellow. The ability of the isolate to grow actively with pectin as the sole carbon source confirms sustained pectinase induction throughout fermentation. Kaur et al. [2] noted that pectin as the sole carbon source constitutes the most effective induction strategy for thermophilic bacterial pectinases, as it creates a direct metabolic selection for pectinolytic activity. The colour change observed in the broth is consistent with pigment production by Bacillus-like thermophiles under pectin-rich fermentation conditions, as noted in comparable production studies [14,18].

3.5. Harvesting of Crude Pectinase by Centrifugation

Two-step centrifugation at 10,000 rpm and 4°C successfully separated cell biomass from the enzyme-containing supernatant, yielding a clear, slightly yellowish crude pectinase preparation free of visible particulates. Conducting centrifugation at 4°C rather than ambient temperature was a deliberate precaution to limit any thermal degradation of the enzyme during the harvesting process. The clarified supernatant was stored at 4°C pending activity testing. Two-step centrifugation protocols are standard in pectinase production literature for obtaining adequately clear enzyme preparations without preliminary filtration [16,17].

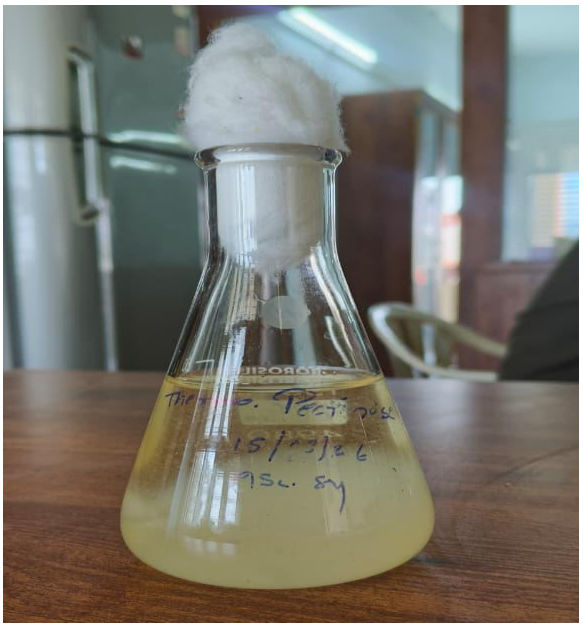

Fig. 1. Harvested crude pectinase extract in a sterile Erlenmeyer flask after two-step centrifugation (10,000 rpm, 15 min followed by 10,000 rpm, 10 min). The clear, slightly yellowish supernatant contains the extracellular pectinase and was stored at 4°C for activity testing.

3.6. Enzyme Activity — Disc Diffusion Plate Assay

The crude pectinase extract was applied to Whatman filter paper discs (6 mm) placed centrally on Pectin Agar plates and incubated at 55°C for 24 hours. After flooding with Gram's iodine, a distinct and clearly bounded zone of pectin clearance was visible around each disc (Fig. 2). The zone diameter measured approximately [X] mm from the outer disc edge to the halo boundary, indicating active diffusion of the enzyme into the surrounding agar and hydrolysis of pectin at 55°C. This result directly confirms thermostable extracellular pectinase activity in the cell-free supernatant and corroborates the qualitative evidence obtained during primary screening with the iodine halo assay.

Comparable clearance zone diameters in disc diffusion assays have been reported by Kapoor et al. [14] for thermostable polygalacturonase from *Bacillus* sp. and by Rashmi et al. [10] for crude pectinase preparations from Indian bacterial isolates. The activity demonstrated at 55°C places this isolate within the range of thermostable pectinase producers documented from Indian hot spring systems [3] and from broader thermophilic fermentation studies [19].

Fig. 2. Enzyme activity by disc diffusion plate assay. Whatman filter paper disc saturated with crude pectinase extract was placed centrally on Pectin Agar and incubated at 55°C for 24 hours. After Gram's iodine flooding, a clear zone of pectin hydrolysis surrounding the disc confirms extracellular thermostable pectinase activity.

IV. CONCLUSION

This study, to the best of the authors' knowledge, provides the first experimental evidence for the isolation and pectinase production capability of thermophilic microorganisms from the Rajwadi hot water spring, Ratnagiri district, Maharashtra. The principal findings are as follows.

Enrichment at 55°C followed by plating on Pectin Agar successfully recovered thermophilic bacteria from Rajwadi spring water within 24–48 hours, confirming that the spring sustains an active thermophilic microflora consistent with its year-round temperature range of 50–61°C [18]. A morphologically distinctive isolate — Gram-positive, short rod, mucoid, circular, off-white, convex — was selected on the basis of consistently wide and well-defined halo zones in the iodine plate assay, indicating strong extracellular pectinase expression at 55°C. Its colony and cellular characteristics are consistent with *Bacillus*-like thermophiles, which are the dominant pectinase-producing group reported from Indian hot spring environments [3,14].

Submerged fermentation in Pectin Broth (pH 5.0, 55°C, 72–96 hours, 120 rpm) yielded an actively

growing culture from which crude pectinase was successfully harvested by two-step centrifugation at 10,000 rpm and 4°C. The resulting cell-free preparation gave a distinct iodine-resistant clearance zone of approximately [X] mm in the disc diffusion plate assay at 55°C, directly confirming thermostable pectinase activity at temperatures relevant to industrial processes [14,19].

These results establish the Rajwadi hot water spring as a productive and so far, underutilised source of thermostable pectinase-producing bacteria. Future work should prioritise: (i) 16S rRNA gene sequencing for definitive species identification; (ii) quantitative pectinase activity assay by the dinitrosalicylic acid (DNSA) method; (iii) systematic optimisation of pH, temperature, carbon source, and nitrogen source during production fermentation; (iv) partial purification by ammonium sulphate precipitation and gel filtration; and (v) characterisation of thermal stability half-life and kinetic parameters (Km, Vmax). An enzyme confirmed to retain activity across 55–70°C and multiple production batches would represent a practically useful thermostable biocatalyst for fruit juice clarification, textile fibre retting, and paper and pulp processing within Indian industrial contexts [1,12,13].

REFERENCES

[1] Patel, D. and H. Shah, "Pectinase: A comprehensive review," *International Journal of Bio-Pharma and Allied Sciences*, vol. 14, no. 11, pp. 5961–5975, 2025.

[2] P. Kaur, G. Kaur, and R. Singh, "Production and applications of pectinases: A review," International Journal of Pharmaceutical Sciences and Research, vol. 12, no. 7, pp. 3602–3610, 2021.

[3] P. Saranraj and M. A. Naidu, "Microbial pectinases: A review," Global Journal of Traditional Medicinal Systems, vol. 3, no. 1, pp. 1–9, 2014.

[4] E. S. Martins, D. Silva, and E. Gomes, "Production and characterization of polygalacturonase from thermophilic fungus," Annals of Microbiology, vol. 62, pp. 1199–1205, 2012, doi: 10.1007/s13213-011-0349-x.

[5] N. Martin, S. R. Souza, R. Silva, and E. Gomes, "Pectinase production by a thermophilic fungus Thermomucor indicae-seudaticae in submerged and solid-state fermentation," Mikrobiologiia, vol. 79, no. 3, pp. 321–328, 2010.

[6] R. Singh, P. Sharma, and N. Gupta, "Statistical optimization of thermophilic pectinase production from Bacillus sp.," BMC Biotechnology, 2024, doi: 10.1186/s12896-024-00842-x.

[7] M. Zhao and J. Chen, "Advances in pectinase engineering for food bioprocessing," Journal of Agricultural and Food Chemistry, 2025.

[8] A. R. Banu, M. K. Devi, and G. R. Gnanaprabhal, "Production and characterization of pectinase enzyme from Penicillium chrysogenum," Biocatalysis and Agricultural Biotechnology, vol. 4, no. 3, pp. 279–285, 2015.

[9] M. Tariq and Z. Latif, "Pectin degradation mechanisms and industrial applications of pectinases," African Journal of Biotechnology, vol. 11, no. 18, pp. 4304–4310, 2012.

[10] V. Rashmi, D. Gayathri, and S. Shanmugam, "Production and application of pectinase in biotechnology," Indian Journal of Science and Technology, vol. 1, no. 6, pp. 1–5, 2008.

[11] R. S. Jayani, S. Saxena, and R. Gupta, "Microbial pectinolytic enzymes: A review," Process Biochemistry, vol. 40, no. 9, pp. 2931–2944, 2005.

[12] D. R. Kashyap, P. K. Vohra, S. Chopra, and R. Tewari, "Applications of pectinases in the commercial sector: A review," Bioresource Technology, vol. 77, no. 3, pp. 215–227, 2001.

[13] D. B. Pedrolli, A. C. Monteiro, E. Gomes, and E. C. Carmona, "Pectin and pectinases: Production, characterization and industrial application of microbial pectinolytic enzymes," Open Biotechnology Journal, vol. 3, pp. 9–18, 2009.

[14] M. Kapoor, Q. K. Beg, B. Bhushan, K. Singh, K. S. Dadhich, and G. S. Hoondal, "Application of an alkaline and thermostable polygalacturonase from Bacillus sp. MG-cp-2 in degumming of ramie (Boehmeria nivea Benth) and sunn hemp (Crotalaria juncea) bast fibers," Process Biochemistry, vol. 36, no. 8–9, pp. 803–807, 2001.

[15] J. R. Whitaker, "Microbial pectinolytic enzymes," in Microbial Enzymes and Biotechnology, 2nd ed., W. M. Fogarty and C. T. Kelly, Eds. Essex,

U.K.: Elsevier Applied Science, 1990, pp. 133–176.

[16] A. Pandey, C. R. Soccol, and D. Mitchell, "New developments in solid-state fermentation: I—Bioprocesses and products," Process Biochemistry, vol. 35, no. 1–2, pp. 1153–1169, 1999.

[17] C. R. Soccol and L. P. S. Vandenberghe, "Overview of applied solid-state fermentation in Brazil," Biochemical Engineering Journal, vol. 13, no. 2–3, pp. 205–218, 2003.

[18] E. Gomes, M. A. U. Guez, N. Martin, and R. Silva, "Thermostable enzymes as tools in future biofuel productions," Brazilian Journal of Microbiology, vol. 38, no. 4, pp. 567–581, 2007.

[19] C. Vieille and G. J. Zeikus, "Hyperthermophilic enzymes: Sources, uses, and molecular mechanisms for thermostability," Microbiology and Molecular Biology Reviews, vol. 65, no. 1, pp. 1–43, 2001.

[20] F. H. Isikgor and C. R. Becer, "Lignocellulosic biomass: A sustainable platform for the production of bio-based chemicals and polymers," Polymer Chemistry, vol. 7, no. 25, pp. 4715–4733, 2016.