**Purification and characterization of β-mannanase produced by *Bacillus cereus* isolated from termite guts**

**Abstract**

Termites are highly effective at breaking down lignocellulose. This study was aimed at isolating β-mannanase producing bacteria from termite guts, and to purify, and characterize the β‑mannanase produced. The termites were isolated from Ikeji environs. Of eight isolates isolated and screened, *Bacillus cereus* showed better potential. The enzyme was produced using saw dust as substrate in a one-factor approach method. The most promising strain, identified as *Bacillus cereus* (accession number MW911450.1), exhibited the highest β-mannanase activity and was selected for further investigation. Purification of the enzyme led to an increase in specific activity from 0.89 to 10.0 mg/mL and a purification fold rise from 1 to 11, accompanied by a decrease in protein content from 56.5 to 3.4 mg/mL using Sephadex G-100. Optimal enzyme activity was observed at temperatures of 25°C and 30°C, with reduced activity at higher temperatures, notably 78% at 90°C. The enzyme showed peak production at pH 4, followed by a steady decline at higher pH levels, with the lowest activity at pH 11. The enzyme retained partial activity in the presence of formaldehyde but was destabilized by solvents such as DMSO, Tween 20, acetone, Triton X, and acetic acid. Inhibitor testing revealed that compounds like urea, sodium A, EDTA, and cysteine enhanced enzyme activity, while SDS inhibited it. Metal ion analysis showed that K⁺, Na⁺, Mg²⁺, and Zn²⁺ promoted activity, with K⁺ having the strongest effect, whereas Mn²⁺ had the least. Substrate concentration had a positive linear effect on enzyme activity, peaking at 0.5 mg/mL. The enzyme’s resilience to alkaline conditions and heat makes it attractive for breaking down hydraulic‑fracturing fluids in oil drilling, pulp bio‑bleaching in papermaking, and scouring/desizing steps in textile processing.

Keywords: β-mannanase, termite guts, lignocellulose, *Bacillus cereus,*  Purification and characterization

**1.0 Introduction**

Lignocellulosic biomass—chiefly cellulose, hemicellulose, pectin, and lignin—accounts for roughly 70 % of plant dry matter (Huy, 2017). Although it represents an abundant carbon feedstock for biofuel production, its practical use is hampered by the expensive pretreatment steps required to release fermentable sugars from the recalcitrant lignocellulosic matrix (Nidhi et al., 2020).

β‑1,4‑D‑mannan polysaccharides—major hemicellulose constituents—are broken down in nature by a mixed team of bacterial and fungal β‑1,4‑D‑mannanases. These enzymes (EC 3.2.1.78) hydrolyze internal β‑1,4 linkages, generating β‑1,4‑manno‑oligosaccharides, mannose, galactose and glucose. Because they underpin applications ranging from pulp bio‑bleaching and feed supplementation to detergent, food, pharmaceutical, textile processing and second‑generation biofuel production (Cheng et al., 2016; Dawood & Ma, 2020), β‑mannanases are attracting intense industrial interest. Current research therefore scouts a broad array of biological sources—plants, animals, bacteria, actinomycetes and fungi—for new catalysts that remain active across wide pH ranges and at elevated temperatures (Moreira & Filho, 2008; Badejo et al., 2021). Within this search, bacterial β‑mannanases are particularly prized for their environmentally benign production, high extracellular yields, cost‑effective purification and outstanding catalytic performance (Cheng et al., 2016).

β‑Mannanase is a mannan‑degrading enzyme, most abundantly sourced from microorganisms—especially bacteria. In animal‑feed formulations it is prized for upgrading ingredients rich in mannans (e.g., hardwood/softwood residues and palm‑kernel meal). By hydrolyzing the β‑1,4‑glycosidic bonds of mannan, glucomannan, and galactomannan, the enzyme releases mannose, glucose, and galactose, thereby aiding hemicellulose breakdown (El‑Sharounya et al., 2015).

Microbial β‑mannanases dominate commercial supply; examples include the enzyme from *Bacillus subtilis* TJ‑102 (Wang et al., 2013). Many mannanase‑producing microbes inhabit mannan‑rich niches such as the termite hind‑gut (Mairizal & Marlida, 2018). Classified as EC 3.2.1.78, β‑mannanases cleave internal β‑(1→4) mannosidic linkages to generate manno‑oligosaccharides (Srivastava & Kapoor, 2017); for glycoside‑hydrolase families 5, 26, and 113, the smallest effective substrates are mannobiose (M2) or mannotriose (M3) (Song et al., 2018).

β‑Mannanase is a carbohydrase that depolymerizes hemicellulosic mannans—galacto‑, gluco‑, and galactoglucomannans included—by hydrolyzing their internal β‑1,4‑mannosidic bonds, releasing mannooligosaccharides (MOS) (Moreira & Filho, 2008). Beyond feed applications, the enzyme underpins processes in the pulp‑and‑paper, detergent, food, oil‑and‑gas, and pharmaceutical sectors. In drug‑delivery, for example, it controls release rates from cross‑linked galactomannan matrices (Japlin, Poernomo & Isnaeni, 2020). Its capacity to upgrade both food and non‑food products has been documented by Chauhan et al. (2012).

To satisfy rising demand, β‑mannanase production has shifted toward microbial platforms. While plants and animals synthesize the enzyme naturally (Song et al., 2018), microorganisms give higher productivities and shorter cultivation cycles—and can be genetically optimized for yield and stability (Demain & Vaishnav, 2009).

This study was aimed to isolate, produce, and purify β‑mannanase from bacteria isolated from the gut of termites.

**2.0 Materials and methods**

**2.1 Sample collection**

Worker termites of *Nasutitermes coxipoensis* were gathered from colonies around Ikeji community in the raining season of 2023 (September), and placed in ventilated, perforated EDTA bottles. Termite samples were collected using randomized sampling method to locate the presence of mound-building termites and their mounds(Abdelmutalab et al., 2023; Aiki et al., 2021). To immobilize them, the insects were held onto ice for 30 minutes, then surface‑sterilized by a 1‑minute dip in 70 % ethanol. After decapitation, the entire gut was excised with a sterile scalpel and homogenized in 0.1 % peptone by vortexing for 4 minutes under aseptic conditions. The homogenates were kept at 4 °C until further use.

The termite species were primarily identified based on their morphological attributes.

**2.2 Isolation of Bacteria**

The dissected gut contents were first suspended in distilled water, then serially diluted under sterile conditions. Bacteria were isolated by the pour‑plate technique following Olutiola et al. (2000). Briefly, 100 µL from each selected dilution was pipetted onto sterile Luria‑Bertani (LB) agar plates, which were incubated at 37 °C for 48 h. Resulting colonies were counted as colony‑forming units per millilitre (CFU mL⁻¹). Mixed cultures were streak‑purified repeatedly to yield single‑colony isolates. These pure strains were maintained on LB‑agar slants at 4 °C for subsequent work.

**2.3 Identification of the isolate**

Bacterial identification combined colony morphology with Gram staining. Pure cultures (18–24 h old) were taken, and a thin smear was prepared on a grease‑free slide with a drop of sterile water and heat‑fixed. The smear was flooded with crystal violet for 30 seconds, rinsed, treated with Gram’s iodine for another 30 s, and washed again. Decolorization followed with ethanol until the purple hue disappeared, after which the slide was rinsed, air‑dried, and examined under an oil‑immersion (×100) objective (Olutiola et al., 2000).

Upon screening the selected isolate was subjected to molecular characterization.

Deoxyribonucleic acid **(**DNA) extraction was carried out as described by (Kiruthika & Padmanabha, 2018). Cell suspension of bacteria isolates (20Ml/mg) were pulverized in 500 µl Cetyltrimetyl ammonium bromide (CTAB) in a sterile mortar. Then, 75 µl of 10% Sodium deodecyl salt (SDS) was added. It was heated in a water bath at 65oC for 15 min. Proteinase K (10 µl) was added and the mixture was incubated at 37oC. Chloroform (500 µl) was then added and swirled for 5 minutes, it was spinned at 10,000 rpm for 10 minutes. Supernatants were collected into fresh eppendorf tubes, 500 µl isopropanol was added into each tube, and the mixture was kept at -20oC for 1hr. It was then spinned at 10,000 rpm for 10 minutes. Supernatants were decanted and the pellets were washed with 70% ethanol and air dried for 30 to 60 minutes. It was re-suspended in 200 µl of sterile water and stored in Tri Ethylene-diamine tetra-acetic acid (EDTA) solution at -20oC for further analysis.

The 5.8S region of the bacteria DNA was amplified by PCR using universal primers ITS5 5’ TCC TCC GCT TAT TGA TAT GC 3’ (forward) and ITS4 5’ GGA AGT AAA AGT CGT AAC AAG G 3’ (reverse) according to (White et al., 1990). The PCR was carried out in a volume of 20 µL 2x PCR Master mix of 10 µl containing 1.5 picolitre, 25 mM MgCl2, 0.5 picolitre of 10 mM dNTPs (dATP, dGTP, dCTP, dTTP), 1µL ITS5 (Forward) primer of 2.5 µM, 1 µL of ITS4 (Reverse) primer of 2.5 µM, 0.2 picolitre Taq polymerase enzymes, 2.0 µL of each extracted chromosomal DNA and nuclease-free water to make final reaction volume of 20 µL. All the tubes containing the mixture for amplification were serially arranged in a [thermal cycler](http://en.wikipedia.org/wiki/Thermal_cycler) block (NYX Technik Inc; Model ATC401, USA). The amplification reaction was carried out in 35 repeated temperature changes cycles thus; Initialization at 94°C for 2 min, denaturation at 95°C for 60 sec, [annealing step](http://en.wikipedia.org/wiki/Annealing_%28biology%29) at 52°C for 60 sec, extension at 72°C for 60 sec, final elongation at 72°C for 5 min and short-term storage was at 4°C. The DNA amplicons obtained were then purified using Gene Clean II Purification Kit (Bio 101, La Jolla, CA, USA) according to manufacturer’s instructions.

The agarose powder was prepared by preparing 1.5 gram of agarose powder which was dissolved in 100 ml Tris acetic EDTA (TAE) buffer, and placed in a microwave oven o melt. It was allowed to cool to 60°C at room temperature. Then 5 µl ethidium bromide (10 mg/ml) was added to 100ml of the gel solution to make a final concentration of 0.5 µL. The solution was thoroughly stirred to disperse the ethidium bromide, it was then poured into the gel rack with a comb inserted at one side of the gel at about 10 mm from the end of the gel. The gel was left to solidify, then the comb was carefully removed thereby creating wells in the gel.

The gel, together with the rack, was completely immersed into TAE buffer in the electrophoretic tank. The gel was completely covered with TAE, with the wells at the negative electrode. DNA (15 µL) of each samples was delivered into each well using micropipette with the 10kbp hyper ladder DNA marker (Ultra ranger) of known molecular weight at the first well while other DNA extracts were placed into other wells. The lid of the electrophoresis chamber was closed and current of 100V was applied for 30 min. The DNA moved toward the positive [anode](http://en.wikipedia.org/wiki/Anode) due to the negative charges on its [phosphate](http://en.wikipedia.org/wiki/Phosphate) backbone. The colored dye in the DNA ladder and DNA samples acts as a "front wave" that runs faster than the DNA itself. When the "front wave" approached the end of the gel, the current was stopped. The rack was then removed from the TAE solution. The DNA band formed were visualized under ultraviolent light.

Purified DNA amplicons were directly prepared in Taq Dye Deoxy terminator cycle sequencing Kit (Applied Biosystems, Falmer, Brighton, UK), and sequenced in an Applied Biosystems automatic DNA sequencer according to the manufacturer's instructions at the Bioscience Department, International Institute of Tropical Agriculture (IITA), Nigeria. The nucleotide sequences were aligned in NCBI Genbank databaset and were explored for similar nucleotide homology using BLAST at a level of identification of 98 % minimal.

**2.4 Screening of Isolates for β-mannanase Production**

β‑Mannanase producers were detected on a screening agar composed of locust‑bean gum (0.5 %w/v), a mineral mix, peptone (0.075 %w/v), yeast extract (0.05 %w/v), and agar (1.5 %w/v), adjusted to pH 6. After incubation, colonies that secreted the enzyme formed clear halos. To enhance halo visibility, plates were flooded with 2 % Congo red, rinsed with 2 M NaCl, and allowed to stand for 15 min, revealing the clearance zones surrounding β‑mannanase‑positive isolates (Mairizal, Yetti Marlida, 2018).

**2.5 Quantitative screening of β-mannanase producing bacteria**

β‑Mannanase was produced under submerged‑fermentation conditions. A 10 % (v/v) seed culture was added to 500 mL of mineral‑salt medium (MSM) containing 0.5 % locust‑bean gum (LBG), 0.075 % peptone, 0.05 % yeast extract, and a mineral mix (0.14 % (NH₄)₂SO₄, 0.20 % KH₂PO₄, 0.03 % MgSO₄·7H₂O, 0.03 % (NH₄)₂CO₃, 0.03 % CaCl₂, 0.0005 % FeSO₄·7H₂O, 0.00016 % MnCl₂·7H₂O, 0.00014 % ZnSO₄·7H₂O, and 0.0002 % CoCl₂), adjusted to pH 6 following Nagar et al. (2012). The medium (in 250‑mL Erlenmeyer flasks) was sterilized at 121 °C, 1 atm for 15 min, inoculated with the screened isolates, and incubated at 37 °C for 24 h on a rotary shaker (120 rpm). Cultures were then centrifuged (6,000 rpm, 15 min, 4 °C); the resulting supernatant—containing crude enzyme—was harvested for β‑mannanase activity and protein assays.

**2.6 Assay for β-mannanase**

β‑Mannanase activity in the culture supernatant was assayed with locust‑bean gum (LBG) or mannan as substrate. For each test, 0.5 mL of a 1 % (w/v) LBG solution in 0.05 M potassium‑phosphate buffer (pH 6.8) was mixed with 0.5 mL of enzyme sample. Control tubes received sterile water instead of enzyme. The mixtures were incubated at 45 °C for 30 min, after which 1 mL of 3,5‑dinitrosalicylic acid (DNS) reagent was added to stop the reaction. Tubes were heated in boiling water for 10 min to develop colour, then cooled. Absorbance was read at 540 nm against a reagent blank. One unit of β‑mannanase activity corresponds to the quantity of enzyme that releases 1 µmol of mannose per minute under these conditions (Badejo et al., 2021).

**2.7 Protein determination**

Protein contents of enzyme production medium was determined using Bradford method (Bradford, 1976). 50 µL of the sample (aliquot enzyme) was added to 750 µL of distilled water in the test tube followed by the addition 200 µL of Bradford reagent. The reaction mixture was then incubated at room temperature for 10 minutes. The absorbance was measured at 595 nm using spectrophotometer, while the concentration of the protein was extrapolated from the standard curve using serum bovine albumin (BSA)(Kielkopf et al., 2020).

**2.8 Purification of β-mannanase**

The purification process included three steps as ammonium sulfate precipitation, DEAE- Sephacel resin and Sephadel G-100 gel filtration chromatography.

**2.8.1 Ammonium sulphate precipitation**

The clarified enzyme extract was first centrifuged at 5,000 rpm for 15 min. The resulting supernatant was then subjected to step‑wise ammonium‑sulfate precipitation: 0–20 %, 20–40 %, 40–60 %, 60–80 %, and finally 80–100 % saturation. The fraction precipitating between 60 % and 80 % saturation was collected, redissolved in 20 mM sodium‑citrate buffer (pH 6.0), and dialyzed against the same buffer for 4 h at 4 °C with gentle stirring (Adiguzel et al., 2015).

**2.8.2 Anion exchange chromatography**

Following dialysis, the enzyme solution (in 20 mM sodium‑citrate buffer, pH 6.0) was loaded onto a pre‑equilibrated DEAE‑cellulose column (2.5 × 30 cm). The column was rinsed with the same buffer until no protein was detected in the effluent, after which bound proteins were eluted with a 0–1 M NaCl gradient. Fractions (3 mL) were collected at a flow rate of 3 mL min⁻¹, and protein elution was monitored at 280 nm. Each fraction was assayed for β‑mannanase activity using locust‑bean‑gum substrate; active fractions were pooled and stored at 4 °C.

**2.8.3 Gel filtration chromatography**

Active fractions from the DEAE column were pooled, dialyzed, and concentrated with a 10 kDa cutoff Amicon concentrator. The concentrate was then loaded onto a Sephacel G‑100 gel‑filtration column (120 × 1 cm) pre‑equilibrated with 20 mM phosphate buffer, pH 5.0, containing 0.5 M NaCl. After elution with the same buffer (salt gradient set via a gradient mixer), fractions were collected, assayed for β‑mannanase activity, and the active ones were combined, reconcentrated, and stored at 4 °C for subsequent use.

**2.9 Determination of optimum temperature on β-mannanase**

To pinpoint the enzyme’s temperature optimum, reaction mixtures (in 50 mM citrate‑phosphate buffer set to the previously determined optimal pH) were incubated for 45 min at temperatures ranging from 25 °C to 90 °C, after which mannanase activity was measured.

**2.10 Thermostability of purified β-mannanase**

Thermal stability was assessed by pre‑incubating the enzyme in 50 mM citrate‑phosphate buffer at different temperatures for 60 min, then measuring the residual mannanase activity (Sathitkowitchai et al., 2022). For each temperature, the maximal activity observed was set to 100 %, and other values were expressed relative to this baseline.

**2.11 Determination of optimum pH on β-mannanase**

The optimum pH for enzyme activity was determined by incubating the enzyme reaction mixture at pH values ranging from 2.0 to 11.0 under standard assay conditions. The buffers used included 50 mM glycine-HCl (pH 3.0), sodium acetate (pH 4.0 and 5.0), phosphate buffer (pH 6.0 and 7.0), Tris-HCl (pH 8.0 and 9.0), and glycine-NaOH (pH 10.0 and 11.0). The reaction mixtures were incubated for 24 hours at 4 °C. Each buffer was also used to prepare a 1% LBG solution, which served as the substrate for β-mannanase activity assays. Enzyme activity was then measured using the standard assay procedure.

**2.12 Effect of pH stability on β-mannanase**

The stability of β-mannanase at different pH levels was evaluated by incubating the purified enzyme with various pH buffers ranging from 4.0 to 9.0 at room temperature for 2 hours. Samples were taken at 15-minute intervals during this period, and enzyme activity was measured using the standard assay conditions.

**2.13 Effects of metal ions on the activity of purified mannanase**

The impact of different metal ions on mannanase activity was investigated by measuring enzyme activity under optimal conditions. The reaction mixture consisted of 1 mL of purified enzyme, 1 mL of a 5 mM solution of metal ions (ZnCl₂, MgCl₂, MnCl₂, CuSO₄, NaCl, and KCl), and 1 mL of 0.5% substrate.

**2.14 Effects of organic solvents on the activity of purified mannanase**

Mannanase stability was evaluated by incubating the enzyme with various organic solvents in 50 mM citrate‑phosphate buffer at room temperature for 60 min. The solvents tested included acetone (1, 5, and 10 mM), Tween 20 (1, 5, and 10 mM), Triton X-100 (1, 5, and 10 mM), acetic acid (1, 5, and 10 mM), formaldehyde (1, 5, and 10 mM), and dimethyl sulfoxide (DMSO) (1, 5, and 10 mM). The residual enzyme activity was measured using the standard assay procedure described earlier.

**2.15 Effects of inhibitors on the activity of purified mannanase**

Mannanase stability was assessed by incubating the enzyme with various inhibitors at room temperature for 60 minutes. The inhibitors tested included SDS (1, 5, and 10 mM), cysteine (1, 5, and 10 mM), EDTA (1, 5, and 10 mM), urea (1, 5, and 10 mM), and sodium azide (1, 5, and 10 mM).

**2.16 Effect of substrate concentration**

The impact of different substrate concentrations on purified mannanase activity was assessed by incubating varying concentrations of manno-oligosaccharides (ranging from 0.1 to 0.6 mg/mL) with mannanase for one hour under optimal conditions. Mannanase activity was measured using standard methods as outlined earlier. Enzyme kinetic parameters were also evaluated (Priya et al., 2014). The Michaelis–Menten constants, Km and Vmax, were calculated from the Lineweaver–Burk plot (Priya et al., 2014).

**Statistical Analyses**

Experiments were carried out in triplicates to ensure reproducibility of the results. The mean and standard deviation of the data from the experiment was calculated.

**3.0 Results and Discussion**

A total of eight bacterial isolates were obtained from the study, with the isolate exhibiting the highest mannanase activity chosen for further enzyme production. This isolate was identified as a Gram-positive short rod bacterium, specifically *Bacillus cereus*, with the accession number MW911450.1. This is in close agreement with reports which earlier stated that the gut of termites were rich in microbiota with the potential to hydrolyse and degrade the lignocellulosic components of plant materials. This could be due to the nutrient-rich environment occurring in the termites’ habitat (Azizi-Shotorkhoft et al., 2016; Li et al., 2023; Oktiarni et al., 2021; Rajan et al., 2022). Also earlier reports have also documented the high potential of *Bacillus cereus* to extracellularly produce β-mannanase (Azizi-Shotorkhoft et al., 2016; Mairizal, 2018). It also corroborates the reports of Zhou *et al.,* 2018 who reported high mannanase expression by the *Bacillus* genus (Zhou et al., 2018). Findings from this study also showed that submerged fermentation was also favourable for mannanase production. This could be due to the ease of control exerted over the environmental factors required for the optimum growth of *B. cereus* and β-mannanase production and it also allowed control over the agitation speed, pH and temperature of the fermenting medium.

As shown in Figure 1, the β-mannanase enzyme purified from *Bacillus cereus* exhibited an increase in specific activity from 0.89 to 10.0 Mg/mL and a corresponding rise in purification fold from 1 to 11 through successive purification steps (Table 1). During this process, there was a notable reduction in protein concentration, dropping from 56.5 to 3.4 Mg/mL, using Sephadex G-100 for the final purification stage. This increase in purification fold also closely agrees with earlier findings which reported an increase in purification fold (17.89-fold) and specific activity of mannanase production by *Bacillus* species (Sutrisno et al., 2020).

Temperature optimization experiments revealed that the enzyme achieved peak activity at 25°C and 30°C, showing 100% and 90% activity, respectively. Beyond these temperatures, enzyme activity gradually declined, with the lowest activity observed at 90°C (78%), as illustrated in Figure 2. This contrasts sharply with reports of Sutrisino *et al*. (2020) who reported optimal temperature for a thermophilic β-mannanase at 65 ℃ (Djelid et al., 2023; Harnentis et al., 2013; Sutrisno et al., 2020b). This could be due to the denaturation of the enzyme at elevated temperatures (Osesusi et al., 2021).

The optimal pH for β-mannanase production was found to be pH 4, followed by pH 5, with a gradual decline in enzyme activity observed from pH 6 onward, as illustrated in Figure 3. The lowest activity was recorded at pH 11 (85%). The pH optimization results indicated that the ideal pH range for mannanase production was between 4.0 and 6.0. This contrasts with previous findings, such as those by (El-Sharounya et al., 2015), who reported that alkaline conditions favoured β-mannanase production by *B. cereus*. However, these results are more aligned with the study by Suae and colleagues, who found that purified mannanase from *Bacillus* sp. R2AL2A had an optimal activity in acidic conditions, although the enzyme remained stable across both acidic and alkaline pH ranges (Magengelele et al., 2021; Sathitkowitchai et al., 2022).

The effect of temperature on enzyme stability was also evaluated. β-mannanase from *Bacillus cereus* showed increased stability with rising temperatures, maintaining full activity at 80°C under static conditions (Figure 4). However, enzyme activity decreased over time with prolonged incubation. The enzyme exhibited peak activity at 25°C during a 180-hour incubation, while activity declined rapidly when incubated at 90°C. This study also confirmed the thermostability of *B. cereus* β-mannanase at 80°C, aligning with previous reports by (Kim et al., 2018; Zhou et al., 2018), who observed thermostable β-mannanase activity in *Bacillus* species within the 65–90°C range, highlighting their suitability for industrial use. The enzyme’s heat resistance may be attributed to temperature-induced structural and functional modifications in proteins, which influence enzymatic activity rates (Sutrisno et al., 2020a). These results further indicate that the thermostability of *B. cereus* β-mannanase is comparable to that of other highly thermostable β-1,4-mannanases, as reported by (Sakai et al., 2017a).

Figure 5 shows the impact of organic solvents and surfactants on the enzyme's stability. The β-mannanase retained partial activity in the presence of formaldehyde at concentrations of 1, 5, and 10 mM (40%, 20%, and 18% activity, respectively). However, significant reductions in stability were observed when the enzyme was exposed to DMSO, Tween 20, acetone, Triton X, and acetic acid. This contrasts with previous findings where Ao-β-mannanase retained 47%, 45%, and 56% of its original activity in the presence of 10% concentrations of SDS, Triton X-100, and Tween-20, respectively (Rahmani et al., 2017; Sakai et al., 2017b).

Figure 6 illustrates the impact of different inhibitors on the stability of *Bacillus cereus* β-mannanase. The study revealed that compounds such as urea, sodium A, EDTA, and cysteine enhanced enzyme activity, whereas SDS significantly reduced it. This closely disagrees with reports that EDTA and urea had significant inhibitory effect on β-mannanase (Soni et al., 2016).

As shown in Figure 7, the presence of certain metal ions also influenced enzyme stability. Potassium (K⁺) at 5 mM demonstrated the greatest enhancement in activity (90%), followed by sodium (Na⁺), magnesium (Mg²⁺), and zinc (Zn²⁺), while manganese (Mn²⁺) had the least positive effect on β-mannanase activity. This contrasts with the findings of Suae and colleagues, who reported that Cu²⁺ and Mn²⁺ had no significant impact on the activity of purified mannanase (Kim et al., 2018; Zhou et al., 2018). The discrepancy may be attributed to the tendency of heavy metal ions to bind strongly to sulfhydryl (-SH) groups, often resulting in irreversible inhibition of enzyme activity.

There was a steady linear increase in β-mannanase activity with rising substrate concentration. The Lineweaver-Burk plot (Figure 8) showed the relationship between reaction velocity and substrate concentration, indicating a Vmax of 2.5 µmol/min/mL and a Km of 31.18 mM for *B. cereus* β-mannanase. This was quite higher to reported Km values reported for mannanases by (Doetal., 2009; Zhao et al., 2010). This is suggestive of the affinity of substrate to fill half the active sites of an enzyme (Meena et al., 2015; Zhou et al., 2018).

Figure 1: Gel-filtration chromatogram of β-mannanase using Sephadel G-100

**Table 1: Purification profile of β-mannanase**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Purification steps** | **Enzyme activity (U/mL)** | **Protein Conc. (mg/mL)** | **Total Activity (U)** | **Total Protein (mg)** | **Specific Activity (mg/mL)** | **Purification fold** | **Yield (%)** |
| Crude Enzyme | 50.6 | 56.5 | 15180 | 16950 | 0.89 | 1 | 100 |
| (NH4)2SO4 Precipitation | 56.5 | 24.2 | 5650 | 2420 | 2.3 | 2.6 | 14.3 |
| Dialysis | 43.7 | 17.6 | 1748 | 704 | 2.5 | 2.8 | 11.5 |
| Sephadex G-100 | 33.9 | 3.4 | 1695 | 170 | 10.0 | 11.1 | 11.2 |

Figure 2: Effect of temperature on the purified β-mannanase

Figure 3: Effect of pH on the purified β- mannanase activity

Figure 4: Effect of temperature on the stability of purified β- mannanase activity

Figure 5: Effect of organic solvents on the activity of purified β-mannanase

Figure 6: Effect of inhibitors on the activity of purified β-mannanase

Figure 7: Effect of metals ions on the activity of purified β-mannanase

Figure 8: Lineweaver-Burk double reciprocal plot of reaction velocity against substrate concentration for purified β-mannanase.

**Conclusion**

In this study, termite gut was exploited due to their lignocellulosic breakdown potential for mannan-degrading bacteria. *Bacillus cereus* isolated from the gut of termites, demonstrated a comparative β-mannanase activity capable of hydrolysis of lignocellulosic substrates. The purified enzyme demonstrated reasonable enzymatic activity during purification particularly during chromatographic separation. The enzyme also contained significant protein content, specific activity and enzymatic yield. It also demonstrated relative stability across varying temperature and pH regimes. The enzyme activity was also time dependent. The enzyme had relative enzymatic activity in the presence of formaldehyde compared to other solvents and surfactants. The enzyme also showed significant activity in the presence of metal ions. The ability of β-mannanase to remain active and stable under alkaline and high-temperature conditions is a valuable trait for its application in various industries, including the enzymatic degradation of hydraulic fracturing fluids in oil drilling, bio-bleaching in the pulp and paper industry, and enzymatic scouring and desizing in textile processing. Additionally, the β-mannanase produced by *B. cereus*, isolated from the termite gut, exhibited notable stability when exposed to various metal ions and organic solvents.

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