Purification, biochemical characterization, and molecular cloning of cellulase from *Bacillus licheniformis* strain Z9 isolated from soil

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Abstract

**Background:** Cellulose is the most prevalent biomass and renewable energy source in nature. The hydrolysis of cellulose to glucose units is essential for the economic exploitation of this natural resource. Cellulase enzyme, which is largely generated by bacteria and fungus, is commonly used to degrade cellulose. Cellulases are used in a variety of industries, including bioethanol manufacturing, textiles, detergents, drugs, food, and paper. As part of our quest to find an efficient biocatalyst for the hydrolysis of cellulose biomass, we describe the amplification, cloning, and sequencing of cellulase (cel9z) from *Bacillus licheniformis* strain Z9, as well as the characterization of the resulting enzyme.

**Results:** Cellulase was partially purified from *B. licheniformis* strain Z9 using (NH₄)₂SO₄ precipitation and Sephadex G-100 gel column chromatography with 356.5 U/mg specific activity, 2.1-purification fold, and 3.07 % yield. The nucleotide sequence of the cellulase gene was deposited to the GenBank, *B. licheniformis* strain Z9 cellulase (cel9z) gene, under accession number MK814929. This corresponds to 1453 nucleotides gene and encodes for a protein composed of 484 amino acids. Comparison of deduced amino acids sequence to other related cellulases showed that the enzyme cel9z can be classified as a glycoside hydrolase family 9. SDS-PAGE analysis of the purified enzyme revealed that the molecular mass was 54.5 kDa. The optimal enzyme activity was observed at pH 7.4 and 30 °C. The enzyme was found to be strongly inhibited by Mg²⁺ and Na⁺, whereas strongly activated by Fe³⁺, Cu²⁺, and Ca²⁺.

**Conclusions:** *B. licheniformis* strain Z9 and its cellulase gene can be further utilized for recombinant production of cellulases for industrial application.

**Keywords:** *Bacillus licheniformis*, Cellulases, Cloning, Biochemical characterization, Purification

Background

Cellulose is the most prevalent polysaccharide in nature and the primary component of plant cell walls [1]. A linear polymer of β-1,4-linked D-glucose residues makes up cellulose. Developing technologies for successful treatment and usage of cellulose wastes as inexpensive carbon sources have been of substantial commercial importance. Cellulose is secreted by some bacterial species, like *Acetobacter, Rhizobium, Xanthococcus, Pseudomonas, Azotobacter, Aerobacter*, and *Alcaligenes*, in addition to being abundant in plants [2]. Due to the urgent need for green energy, cellulose has acquired economic interest in its hydrolysis bio-technique in recent decades. One of the most common methods for converting cellulose into reducing sugars, which can then be turned into ethanol and other compounds, is microbial hydrolysis [3].

Cellulases are a family of enzymes that catalyze the hydrolysis of cellulose to liberate glucose units [4]. Endoglucanase (EC 3.2.1.4), exoglucanase (EC 3.2.1.91), and β-glucosidase (EC 3.2.1.21) are the three primary components of the cellulase complex.
enzymes [5]. Endoglucanase works by cleaving intermolecular β-1,4-glycosidic bonds inside the cellulose chain to liberate oligosaccharides for exoglucanase and β-glucosidase to further hydrolyze [6]. Endoglucanase and exoglucanase create cello-oligosaccharides and cellobiose from cellulose, which are then converted to glucose by β-glucosidase [7]. Cellulases derived from fungi, bacteria, and yeasts have been studied extensively [3]. Different bacterial species as Bacillus [8], Clostridium, and Ruminococcus [9] have all been reported to produce cellulases. Cellulases are used in juice extraction processes, pulp and paper, textile industry, secondary metabolites, animal feed, and the production of fermentable sugars for biofuels [10]. Thus, the demand for this enzyme is increasing exponentially [11].

Cellulases were isolated and identified first only from culturable bacteria using a fermentation technique, and the whole cellulase potential of the site was not completely investigated. Due to the high substrate cost necessary for cellulase induction and the problems of maintaining the appropriate conditions for cellulase production, fermentation approaches have limitations [12]. As a result, recent breakthroughs in molecular approaches, such as the production of metagenomic libraries, will expand the pool of cellulolytic enzymes suitable for biofuel research, potentially solving these challenges. This new method will allow the extraction of cellulases and related enzymes from bacteria that are otherwise unculturable and may create novel enzymes with specialized applications [13]. Uncultured microorganisms make up a large part of the natural world’s biodiversity. Only 0.1–1% of the natural environment is made up of microorganisms that can be cultivated using conventional laboratory techniques [14]. Genes derived from metagenomic techniques have proven to be useful in identifying novel genes with specialized functions [15]. A new strategy for discovering novel enzymes is to clone and express the cellulase gene in an efficient host cell like Escherichia coli [16].

Our earlier studies based on isolation and identification of cellulolytic B. licheniformis strain Z9 from soils having the highest cellulolytic activity [17]. The present study is concerned with the amplification, sequencing, and cloning of the gene encoding cellulase. Also, biochemical characterization was investigated to determine the optimum enzyme activity. This research, combined with ongoing expression research, could lead to a low-cost system based on genetically recombinant Escherichia coli with industrial applications.

Methods

Bacterial strain

B. licheniformis strain Z9 (KT693282) was isolated from farm soil at Menoufia governorate, Egypt (30°35’50.09” North, 30°59’15.48” East) [17] and tested for its high performance for cellulase activity. The bacterial strain was cultured in nutrient broth and stored on nutrient agar at 4 °C and as 50% glycerol stocks at −80 °C.

Enzyme assay

The cellulase activity was observed by the 3, 5 dinitrosalicylic acid (DNS) method as described by Miller [18]. CMCase activity was determined by incubating 500 μl of 1% CMC in 50 mM sodium phosphate buffer (pH 7.2) with 500 μl cell free extract for 30 min at 50 °C. The reaction was stopped by adding 1 mL of 3, 5 dinitrosalicylic acid (DNS) reagent and incubated in a water bath for 10 min at 50 °C. After cooling at room temperature, the amount of glucose released was investigated with a spectrophotometer at 540 nm against a blank containing all the reagents minus the crude enzyme. A calibration curve for glucose was constructed to determine the CMCase activity. One unit (U) of cellulase activity was defined as the amount of enzyme that released 1 μmol of glucose per minute under the standard assay conditions. All assays were performed in triplicate.

Protein assay

Protein concentration was estimated by the method of Bradford [19] using bovine serum albumin (BSA) as a standard against a blank was set with only distilled water.

Molecular identification and DNA sequence analysis

To amplify the cellulase gene from the B. licheniformis strain Z9, degenerated gene-specific primers were designed complementary to the B. licheniformis strain SRCM100027 (CP021677) cellulase [ARW53264] gene sequence retrieved from the NCBI nucleotide database. This includes Forward primer Zf1:′5′ATGGCGTTATTCTGCGCAATCCTGTCA-3′) and reverse primer Zr1 (5′ GGCCATGTCGCTCTGCACGTAAGTGG-3′). The PCR amplification reaction was performed in a total volume of 50 μl containing 2 μl of template DNA (50 ng/μL), 25 μl of 2X Taq PCR Master Mix (contains Taq DNA polymerase (0.05 U/μL), reaction buffer, 4 mM MgCl2, and 0.4 mM of each dNTP) provided by Thermo Fisher Scientific, USA, 2 μl of forward primer, 2 μl of reverse primer and 19 μl of Nuclease-free water. The following PCR conditions were used for amplification of cellulase gene: initial denaturation at 94 °C, 5 min, and 35 cycles of the following steps: denaturation at 94 °C, 30
s; annealing at 55 °C, 30 s; extension at 72 °C, 1 min; and final extension at 72 °C, 5 min. The amplified PCR products were checked on 1% agarose gel stained with ethidium bromide and visualized on a UV transilluminator. The purified PCR products were cloned into pSC-A-amp/kan PCR Cloning Vector as recommended by the manufacturer (Stratagene, Agilent Technologies, USA). StrataClone SoloPack competent cells were used for the transformation and recovery of high-quality recombinant DNA. The purified PCR products were Sanger-sequenced with the BigDye terminator v3.1 sequencing kit and ABI PRISM® 3730xl Analyzer capillary sequencer (Applied Biosystems, Foster City, CA). Nucleotide sequences were determined on both strands of PCR amplification products at Macrogen Company, Seoul, South Korea. The nucleotide sequence data was assembled, analyzed with GENETYX computer software (Software Development Co. Ltd., Tokyo, Japan). The consensus sequence obtained was compared with other sequences available in the GenBank/NCBI database using the BLAST tool [20] and aligned using CLUSTAL O [21]. The sequence was deposited with the GenBank Data Library under accession number MK814929. The deduced amino acids sequence was analyzed with UniprotKB database Release 2020, Washington, USA (http:// UniprotKB.org/). The phylogenetic tree was drawn with MEGA11 software [22], using Kimura’s two-parameter model of sequence evolution. The robustness of the phylogenetic tree was estimated via bootstrap analysis using 1000 resampling.

Conserved domain analysis and hydropathy plots of predicted cellulase cel9z
The protein sequences of cellulase cel9z were subjected to conserved domain analysis using the Conserved Domain Database tool of NCBI [23] (http://www.ncbi.nlm.nih.gov/Structure/cdd/). A hydropathy plot was generated using Expasy-Protscale (https://web.expasy.org/protscale/).

Preparation of cell extracts
Cellulase enzyme was produced under submerged fermentation from isolated B. licheniformis strain Z9. Conical flasks containing 50 mL of a carboxymethylcellulose medium [24] were supplemented with 0.5% (w/v) of CMC at pH 7.2. The flasks were autoclaved at 121 °C for 20 min. These sterilized flasks were inoculated with 10% of an inoculum culture of B. licheniformis strain Z9 and incubated under agitation at 150 pm, for 72 h at 30 °C according to the method of MARCO [25]. Then, the culture was centrifuged at 10,000×g for 15 min at 4 °C to separate the cells. The clear cell-free supernatant (crude extract) was collected and concentrated by ultrafiltration using a MILTEX-HV ultrafiltration cell (Millipore, Ireland). The crude extract was stored at 4 °C and used for further analyses [26].

Purification of cellulase
All steps were performed at 4 °C unless otherwise noted. The crude extract was saturated with (20–80%) ammonium sulphate with continuous stirring at 4 °C followed by centrifugation at 10,000×g for 15 min. Ammonium sulfate fraction (the developed pellet) was dialyzed against 50 mM sodium phosphate buffer (pH 7.2) for 6 h at 4 °C in a dialysis bag (20,000 kDa) and immersed in the same buffer at 4 °C overnight. Changing buffer at every 1 h intervals is important to achieve proper purification [27]. Fractions with high activity of cellulase were pooled together, dialyzed towards the above buffer, and concentrated by lyophilization (− 50 °C) for the next purification step. The dialysate was loaded onto a Sephadex G-100 column (2.5 × 40 cm) equilibrated with 50 mM sodium phosphate buffer (pH 7.2), and eluted in a gradient of NaCl (0–1 mol L⁻¹) [25]. The cellulase was eluted from the column at a flow rate of 5 ml/min using the same buffer. Thirty-five fractions (5 ml each) were collected, dialyzed against the same buffer and the protein content was measured with a spectrophotometer at 280 nm. Fractions were checked for their purity by applying sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) technique.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis (SDS-PAGE)
To estimate the molecular weight of the partially purified enzyme, SDS-PAGE was done as reported by Laemmli [28]. Briefly, the SDS-PAGE gel slabs were prepared with upper 4% stacking gel and lower 10% resolving gel using a Bio-Rad electrophoresis system (Bio-Rad, CA, USA). The protein samples were mixed with sample buffer containing 62.5 mM Tris–HCl, pH 6.8, 25% glycerol, 0.01% bromophenol blue, 2% SDS, 10% β-mercaptoethanol, and then heated for 3 min before loading to the gel. The electrophoresis was carried out in running buffer (0.25 M Tris, 0.192 M glycine, 0.1% SDS, pH 8.3) and the gel was then stained by a solution of 0.15% Coomassie Brilliant Blue (CBB) R-250 in 50% ethanol and 10% glacial acetic acid. The samples were dissolved with sample buffer (50 mM Tris–HCl pH 6.8, 2% SDS, 10% glycerol, 1% β-mercaptoethanol, 0.01% bromphenol blue) and then applied to the wells, resolved by applying a constant current (100 V) across the gel. After the run, the resolved bands were visualized by Coomassie brilliant blue R-250 staining method. The molecular weights were estimated by comparing with standard broad range protein marker.
appropriate concentration of enzyme was added to 100 μL of purified cel9z protein. One hundred microliters of the appropriate concentration of enzyme was added to 100 μL of 1% CMC and completed to 1 mL with 800 μL of various buffers. The buffers were citrate phosphate (pH 4.2–7), Tris (Hydroxymethyl) aminomethane (Tris) (pH 7.2–9.0), and glycine-NaOH (9.5-10.6), and the activity was measured as described by Miller [18]. The non-treated enzyme activity was regarded as control (100%).

Statistical analysis
Results were expressed as mean ± standard deviation and the data was analyzed using one-way ANOVA using GraphPad Prism for windows, www.graphpad.com.

Results
Molecular identification of cellulase gene
A total volume of 50 μL of PCR reaction was used for amplification of cellulase cel9z from B. licheniformis strain Z9. The length of the fragments was about 1500 bp. The PCR products of strain Z9 were sequenced to obtain an open reading frame (ORF) of 1453-bp gene sequence, which was consistent with the result of electrophoresis. This band was then excised, eluted, purified, and then subjected to cloning.

The phylogenetic analysis of the cel9z and its related hits was carried out. Analysis revealed that the isolated B. licheniformis strain Z9 cellulase (cel9z) gene formed a distinct clade, containing endoglucanase and cellulase of B. licheniformis (Fig. 1A).

Nucleotide sequence analysis
The obtained sequence was analyzed and compared with a sequence in the nucleotide database (NCBI) using the BLAST algorithm. The 1453 bp fragment of cel9z was fully sequenced at Macrogen Company, Seoul, South Korea, encoding a protein of 484 amino acids, with a calculated molecular mass of about 54.4 kDa as shown in Supplementary data 1.

Analysis and alignment of cel9z protein sequences
The deduced amino acid sequence encoding cel9z was conducted from the sequence of nucleotides data. The amino acid sequence was aligned with the amino acid sequences of other organisms using UniProtKB program. The results
obtained were shown in Supplementary data 2. The amino acid sequence comparison against protein databases indicated that (cel9z) shared over 97% similarity with their homologs. The highest sequence identity of cellulase (cel9z) was 99.7% that compared with the Endoglucanase A of \( B. licheniformis \) (accession number TWK88936) which was defined by the whole genomic sequence.

Conserved domain analysis of cellulase cel9z protein revealed the presence of glycosyl hydrolase 9 domain of glycoside hydrolase 9 superfamily in the protein. The domain analysis found a specific hit for the with e value at 2.12e−165. Consequently, there was high confidence in the association between the protein query sequence and a conserved domain, resulting in a high confidence level for the inferred function of the protein query sequence. Further, hydropathy plots indicated that the protein was hydrophilic with a hydropathy score of −3.

**Enzyme purification**

The purification profile of cellulase (cel9z) of \( B. licheniformis \) were listed in Table 1. Ammonium sulfate precipitation gave a purification fold of about 1.96 with a specific activity of 333.4 U/mg and 51.1% yield, while purification fold of about 2.1 and specific activity of 356.5 U/mg with 3.07% yield was achieved for gel filtration chromatography (Fig. 2A).

**SDS-PAGE and zymogram analysis**

Enzyme activity is concentrated and subjected to SDS-PAGE. The enzyme cel9z showed a single band on

![Fig. 1](image_url)

**Table 1** Overall purification profile of cellulase from \( Bacillus licheniformis \) strain-Z9

| Purification step                              | Volume (ml) | Total protein (mg) | Total activity (units) | Specific activity (U mg\(^{-1}\) Protein min\(^{-1}\)) | Purification (fold) | Yield (%) |
|-----------------------------------------------|-------------|--------------------|------------------------|-------------------------------------------------------|---------------------|-----------|
| Crude extract                                 | 150         | 157.5              | 26700                  | 169.5                                                 | 1                   | 100       |
| (NH\(_4\))\(_2\)SO\(_4\) Precipitate          | 39          | 40.95              | 13650                  | 333.4                                                 | 1.96                | 51.1      |
| Dialysis                                      | 20          | 9.5                | 3300                   | 347.3                                                 | 2.04                | 12.35     |
| Gel exclusion chromatography (Sephadex G-100) | 15          | 2.3                | 820                    | 356.5                                                 | 2.1                 | 3.07      |

\(^{a,b}\) values represent the mean of the values from three independent experiments, with a standard deviation of <±5%

Specific activity = total activity/total protein

\(^{c}\) Purification fold = specific activity of particular purification step/specific activity of the crude enzyme

\(^{d}\) Yield (%) = (total activity of the particular purification step/total activity of the crude enzyme) × 100
SDS-PAGE with a molecular weight of approximately 54.4 kDa for both crude extract and partially purified enzyme. The enzymatic activity of partially purified cel9z is confirmed by CMC zymographic analysis. It is shown as a yellow halo against a red background (Fig. 2B).

Enzyme activity was concentrated and subjected to SDS-PAGE. The enzyme cel9z showed a single band on SDS-PAGE with a molecular weight of approximately 54.4 kDa for the partially purified enzyme. The enzymatic activity of purified cel9z was confirmed by CMC zymographic analysis. It was shown as a yellow halo against a red background (Fig. 2B).

Biochemical characterization of cel9z

Screening of optimal temperature

The effect of temperature on the enzyme activity of cel9z from B. licheniformis was determined at various temperatures ranging from 10 to 60 °C as shown in (Fig. 3A). Results demonstrated that the optimum temperature of the enzyme was around 30 °C.

Screening of optimal pH

The effect of pH on the enzyme activity of cel9z from B. licheniformis was also examined at various pH levels ranging from pH 4.3 to pH 10.5. Cel9z exhibited the highest activity at pH 5.5–8, with an optimum pH of the enzyme activity at pH 7.4 (Fig. 3B).

Screening of the effect of metal ions and chemical reagents

The effect of metal ions and some chemical reagents at a final concentration of 1 mM (Ag⁺, Na⁺, Cu²⁺, Co²⁺, Ca²⁺, Fe³⁺, Mg²⁺, SDS, and EDTA) on cel9z enzyme activity from B. licheniformis strain Z9 was studied. The enzyme activity was strongly inhibited by Mg²⁺ and Na⁺ between 31 and 53.8% whereas Fe³⁺, Ca²⁺, and Cu²⁺ significantly activated the enzyme activity between 98.4 and 83.2% (Fig. 3C). Enzyme activity demonstrated a
decrease by the chemical reagent EDTA by 40.3%, while SDS exhibited activation for the enzyme by 92%.

Discussion
The increased demand for finding new sources of biofuels and renewable energy as an alternative to fossil fuels is one of the most common interests of researches worldwide. The enzymatic hydrolysis of cellulosic biomass by cellulases has been increased in several studies for their employment in many industrial applications. Different Bacilli species presented relevant results related to cellulase production [30]. In this study, gene encoding cellulase (cel9z) was derived from B. licheniformis strain-Z9 and was successfully amplified, purified, and sequenced. Cloning of powerful cellulase genes might be very important for the successful production and consequently industrial application of the enzyme. Furthermore, researchers have concentrated on producing recombinant cellulase. As a host, E. coli does not require any special media and grows rapidly. The key benefit of recombinant cellulase is that it may be scaled up to commercial levels without the usage of expensive substrates [31]. Cel9z from B. licheniformis strain Z9 has been cloned into the pSC-A-amp/kan PCR Cloning Vector, and further research into cel9z in a recombinant expression system is underway.

To provide pure and homogeneous cellulase for industrial applications, a comprehensive purification process is required. Therefore, the purification of crude enzyme extracts from B. licheniformis strain-Z9 was achieved through the combination of ammonium sulphate precipitation, 40–80% saturation followed by gel filtration on Sephadex G-100 with a total yield of 3.07% and 2.1-fold purification. Enhancement of specific activity is observed in each of the purification steps. In this respect, Azadian et al. [24] reported overall purification fold of the enzyme about 8.85 with the specific activity of 412.32 U/mg of cellulase from Bacillus sp. The purified enzyme was emerged as only one protein band with a molecular mass of 54.4 kDa on sodium dodecyl sulfate-polyacrylamide gel electrophoresis, confirming its purity.

The molecular weight 54.4 kDa of the partially purified enzyme is close to that reported by Assareh et al. [32]. Our results are also similar to those obtained by Rawat and Tewari [8] where cellulases of Bacillus sp. [33]. Similar results were obtained by Aygan et al. [34] who observed endoglucanases activity for the genus Bacillus at pH values from 8.5 to 10.0. In contrast, other studies have reported that the optimum pH for purified
cellulase from *B. circulans* was 4.5 [35] while cellulase was produced by *Bacillus* sp. C14 was 11 [36].

The pH of the growth medium influences many enzymatic reactions by affecting the transport of chemical products and enzymes across the cell membrane [37]. A wide pH range is required for the application of enzymes in numerous conditions [3]. In the present study, the optimum pH was 7.4 and it was active over the broad pH region of 5.5–8. Obtained results are in good agreement with Pokhrel et al. [36] who reported that the optimum pH for the enzyme of *Bacillus subtilis* ranged between 6.5 and 7.5. Also, the optimum pH for *B. licheniformis* NLRI-X33 was 7.5 [38]. Other similar findings by Ekwealor et al. [39] showed very good activity at pH range 6 to 9.

The optimum temperature of the partially purified cellulase was found to be around 30 °C. The results are in close agreement with the study of *B. licheniformis* isolated from compost by Nallusamy et al. [40] at 37 °C, cellulase activity retained 87% activity. Also, the maximum endo (1,4) β-d-glucanase production from *B. licheniformis* KIBGE-1B2 was observed at 37 °C [41]. The same maximum cellulase activity at 37 °C of *B. pumilus* EWBCM1 isolated from the gut of earthworm was determined by Shankar and Isaiarasu [42]. There is a remarkable decrease of enzyme activity over 45 °C and retained only 42% activity at 60 °C. In this respect, Maurya et al. [43] reported that over a certain temperature, enzyme activity decreases with an increase in temperature because of enzyme denaturation. During the saccharification and fermentation processes, a low-temperature adaptation can save energy and money [3]. As a result, it can be used in a variety of industries, including food, feedstuffs, textiles, and pharmaceuticals [44]. According to current research, cel9z optimal activity occurs at a low temperature, which may be helpful for its prospective application.

The partially purified cellulase was also screened to determine its enzymatic activity in the presence of different metal ions Ag⁺, Na⁺, Cu²⁺, Co²⁺, Ca²⁺, Fe³⁺, Mg²⁺, SDS, and EDTA. The cellulase activity was found to be enhanced in the presence of Fe³⁺, Cu²⁺, and Ca²⁺. However, the enzyme was inhibited by the presence of metal ions Mg²⁺, and Na⁺. The opposite result was reported by Azadian et al. [24], where the CMCase activity was enhanced in the presence of Mg²⁺ (110%). The inhibition by the same divalent cations was also reported in cellulase enzymes from *B. licheniformis* strain MK7 and *Bacillus* amyloquefaciens DL-3 [45]. The inhibitory effect of Mg²⁺ in this study is contrary to the work of Ekwealor et al. [39] who reported the stimulatory effect of MgSO₄ on the activity of CMCase. Reports also revealed that metal ion in the form of a salt such as CaCl₂·6H₂O provides protection to some enzymes against thermal denaturation and plays an important role to stabilize the native forms at high temperatures [32]. The inhibition of cellulase by Mg²⁺ and Na⁺ ions may be linked to the competition between the exogenous cations and the protein-associated cations, resulting in a decreased metalloenzyme activity. EDTA was found to be inhibitory to the activity of cellulase used in this study. Low concentration of low valent metal ions had almost no inhibition effects on enzyme activity. Therefore, employing cel9z in the industry is promising.

Currently, some developed studies were applied to produce industrial cellulase by *Bacilli* strains due to the high bacterial growth, compared to fungi, and their ability to adapt to low-cost carbon sources, such as sugar cane bagasse. In addition, bacterial cellulase is considered a potent enzyme for the application in second-generation ethanol produced from sugarcane biomass in Brazil [46]. Further studies of the cellulase gene (cel9z) will provide insights into the function of the protein and its biochemical properties.

### Conclusion

In the current study, successful cloning of the gene-producing cellulase appears to be a viable technique that will lead to the creation of a low-cost effective strategy for achieving considerable lignocellulosic waste bioconversions. Furthermore, the purified cellulase (cel9z) from *B. licheniformis* Z9 showed a wide pH and temperature range. Further expression of the cloned gene will reveal information regarding the function of the produced protein, as well as its biochemical properties and prospective industrial applications.

### Abbreviations

BSA: Bovine serum albumin; Cel9z: Cellulase of *Bacillus licheniformis* strain Z9; CMC: Carboxymethylcellulose; DNA: Deoxyribonucleic acid; DMS: Dimethyl sulfoxide; EDTA: Ethylenediaminetetraacetic acid; ORFs: Open reading frame; PCR: Polymerase chain reaction; SDS-PAGE: Sodium dodecyl sulphate-polyacrylamide gel electrophoresis; 16S rRNA: 16S ribosomal ribonucleic acid.

### Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43141-022-00317-4.

**Additional file 1: Supplementary data 1.** The nucleotide sequence of *Bacillus licheniformis* strain Z9 recombinant cellulase (cel9z) gene and its deduced amino acid residues. **Supplementary data 2.** Multiple sequence alignment of *Bacillus licheniformis* (Cel9z) with other glycosyl hydrolase family QGA89116. B. licheniformis (Cel9z) deduced amino acid sequence. ARW53264: Cellulase [B. licheniformis]; NVB33365: endoglucanase [B. licheniformis]; WP_217903639: glycoside hydrolase family 9 protein [B. licheniformis]; QGA89116: B. licheniformis (Cel9z) deduced amino acid sequence. ARW53264: Cellulase [B. licheniformis]; NVB33365: endoglucanase [B. licheniformis]; WP_217903639: glycoside hydrolase family 9 protein [B. licheniformis]; QGA89116: B. licheniformis (Cel9z) deduced amino acid sequence.
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