Cloning of cellulase gene using metagenomic approach of soils collected from Wadi El Natrun, an extremophilic desert valley in Egypt

Safaa M. Ali1,2*, Nadia A. Soliman3, Samia Abd Allah Abdal-Aziz1 and Yasser R. Abdel-Fattah3

Abstract

Background: Wadi El Natrun microorganisms have been considered as a new resource for natural products due to its extreme condition of salinity and alkalinity. Therefore, this study was devoted to generate metagenomic library from soils collected from such an extreme environment in order to clone a novel cellulase for physique industrial applications.

Results: Total soil-DNA was successfully extracted, and then digested by different restriction enzymes. Purified fragments ranged ~ 200–6500 bp were ligated and were cloned into plasmid cloning vector (pUC19) by using Escherichia coli DH5α (E. coli) host cells. A constructed metagenomic library composed of 270 clones was screened on carboxymethylcellulose (CMC) agar plate where the active clones had been characterized by the formation of the yellowish halo zone. Thereafter, clone 1 was selected as the most active as being based on cellulase activity quantification (19 μ/ml). Plasmid related to clone 1 encoded cellSNSY gene of approximately 1.5 kb was subjected to molecular characterization; the obtained partial sequence of 861 bps encoded 287 amino acids showing 76% similarity to the endoglucanase gene of Bacillus amyloliquefaciens. The recombinant cellSNSY was expressed under lacZ promoter at 1 mM of isopropyl β-d-1-thiogalactopyranoside (IPTG), giving 21 μ/ml cellulase after ~ 27 h. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and an activity staining of the recombinant cellSNSY which revealed an active band with a molecular mass ~ 59 kDa appeared in the induced sample. The maximum enzyme activity of crude cellSNSY was observed at 45 °C and for a pH of 8.5. Interestingly, the enzyme activity was slightly inhibited by ethylenediamine tetraacetic acid (EDTA) and methanol. It showed high resistance to the tested heavy metals and the surfactant which ordered Zn > (SDS,Fe)>Mn>Cu.

Conclusions: This study established an easy and a skillful way to clone/express a new found cellulase gene(s) under lacZ promoter. The isolated recombinant cellSNSY showed 76% similarity to endoglucanase gene, and the enzyme showed tolerance to the mostly tested agents including heavy metals, surfactant, solvents, and EDTA. Additionally, the studied recombinant showed a high stability up to 55 °C and for alkaline pH 8.5. These features make it an ample and viable for many applications.

Keywords: Cellulase, Cloning, Degradation, Expression, Extremophilic, Metagenome

*Correspondence: Safaa.mohamedali@yahoo.com
2 Present address: City of Scientific Research and Technological Applications (SRTA-City), New Burg El-Arab City, Universities and Research Institutes Zone, Alexandria Post 21934, Egypt
Full list of author information is available at the end of the article

© The Author(s) 2022. Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

Background

Cellulose is the most cost-effective, natural, and renewable organic compound used as a carbon source in an assortment of solicitations [1]. The much more common carbohydrate on the planet is cellulose. It is the primary
component of plant materials. It can be found in a multiplicity of dwellings including wood and plant cell walls, bacteria, algae, and tunicates which are the only ones that have it. This abundant supply naturally enables the development of novel customs for this adaptable material [2]. It is an extensive chain of glucose units linked by 1,4-glycosidic connections [3, 4]. Cellulosic materials have been used for a number of devotions in recent decades including biomedical applications [5, 6].

Chemical and enzymatic hydrolysis are the two methods for cellulose to glucose conversion (Cellulases are used as an enzymatic method for this resolution and are known as an eco-friendly process because it does not produce secondary polluting metabolites) [7–9]. Cellulases, in specific, are accountable for cellulose decomposition by hydrolyzing the 1,4-glycosidic linkages [10]. Cellulases are one of the greatest recurrently used industrial enzymes with over 30 years of commercial handiness [11]. These are inducible enzymes bent by a variety of microorganisms, such as bacteria and fungus, when growing on cellulose materials. Cellulase is a multifunctional enzyme that combines exoglucanase, endoglucanase, and β-glucosidase to make a public announcement little expanse of glucose. As a result, cellulose is converted to glucose, a simple sugar that can be fermented into cellulosic biofuels [12]. Animal and plant enzymes are less stable than microbial enzymes. They have a number of advantages including the aptitude to be manufactured at a lower cost and in a shorter time frame using fermentation processes with high consistency, as well as the ability to readily optimize the process [13]. As a result, cellulases enzymes are an option in a wide range of industrial situations including the paper and the pulp industries, textile and bioethanol industries, wine and brewery industry, food industries, animal feed industry, agricultural and detergent industries [11, 14–16], pharmaceutical industries, and the handling of waste [17, 18].

In biotechnology, extremophile bacteria have a wide range of current and potential applications. The diversity of prokaryotic populatons was investigated in the water and from the sediments of three largest lakes in the Wadi El Natrun [19].

The scarcity of novel cellulases with diverse features has evolved into a bottleneck in the effectual cellulase use. The term “metagename” refers to a group of genes discovered in the environment with foreseeable vast repositories of cellulases. New cellulases, on the contrary, are difficult to discover by using functional metagenomic library screening. Since the technique is based on the cellulase activity rather than on the sequence similarity. Consequently, finding new cellulases via functional screening of metagenomic libraries is difficult in nature. The targeted selection of novel cellulase sequences for high-throughput expression is possible with metagenomic sequencing. Next-generation sequencing, such as metagenomics, has revolutionized the study of the “unseen majority.” Metagenomics application accustomed to investigate new enzymes is important to result for permitting researchers to get the knowledge about the diversity of the microorganisms with reaching for 99% and for numerous sorts of genes coding catalyst that have not nonetheless been known [20, 21]. Metagenomics has been thought-about as an economical approach for locating novel cellulase/hemicellulase from microbes, particularly ingenious microbes [22]. This method allows us direct access to bioactive candidates as well as in-depth study of microbial genomes. The current metagenomic research of new enzymes and systems that serve as hosts opens a Pandora’s Box for improving the bioenergy industry, which plays a significant part in the bio-economy [23].

The objective of the present study aimed to clone functional gene(s) degrading cellulose using soil-metagenomics from Wadi El Natrun located in Egypt due to its extreme conditions. The process started by soil collection, total deoxyribonucleic acid (DNA) isolation, fragmentation, cloning, and then, expression of the isolated gene(s) in a suitable host cells (E. coli DH5α). Subsequently, molecular and functional characterization for cloned gene was investigated.

Methods

Sample collection and preparation
The soil samples (2) were collected from Alexandria (2018), Egypt’s Wadi El Natrun (Latitude & Longitude (WGS84): 30° 27′ 20″ North, 30° 10′ 20″ East), secondly were transferred to the laboratory in ice box, thirdly were placed in a deep freezer (−20 °C), and then were inspected.

Chromosomal DNA preparation
DNA isolation from soil sample was carried out using soil-isolation DNA kit (QIAGEN). In brief, the soil sample (500 mg) was suspended in 10 ml of 0.1 M sodium phosphate buffer (pH 8) before being added to 0.5 ml of 20% sodium dodecyl sulfate (SDS) and was mixed numerous times by inversion. Afterwards, 20 μl lysozyme (10 mg/ ml) was added and was mixed well (30 s); then the protocol of DNA isolation was completed according to the manufacturer’s recommendation. Afterwards, the purity and the concentration of the isolated DNA were detected by using NANO DROP (Thermo Scientific™ NanoDrop 2000).
DNA restriction digestion
The isolated soil-DNA was digested in 20 μl reaction volume; 2 μl of enzyme buffer and 1–2 U restriction enzyme were added. DNA digestions with restriction enzymes were performed under the reaction conditions specific for each enzyme, as being suggested by the manufacturers (Fermentas). Different restriction enzymes were used (BamHI, EcoRI, HindIII, and SalI) for the DNA digestion and the cloning vector (pUC19), as well.

DNA ligation
A ligation of the digested DNA-vector (ratio 4:1) was made into 20 μl reaction volume using T4 DNA ligase (1U) (Fermentas) and 2 μl buffer as the mixture reaction was kept at 16 °C overnight. PUC 19 (plasmid cloning vectors) was used as cloning and expression vector which allowed blue/white screening for the recombinant clones through LacZ promoter in the presence of the lactose analogue (IPTG, 1M) and the chromogenic agent 5-bromo-4-chloro-3-indolyl-β-d-galactopyranoside (X-gal, 200 mg/ml).

Competent cells preparation and transformation
Escherichia coli DH5α was prepared according to Sam-brock et al. [24]; 100 ml Luria-Bertani (LB,low salt) liquid medium of the following composition (g/l): yeast extract, 10; peptone, 5; NaCl, 5; they were inoculated with 100 μl overnight culture of E. coli DH5α and were kept warm at 37 °C with shaking (170 rpm) till optical density (OD 600) 0.6–0.7. The flask was ice-chilled and was aliquoted into 50 ml portions. Cells were collected with centrifugation at 4000 rpm and re-suspended in one ml of transformation storage solution (TSS). This solution composed of (w/v %) polyethylene glycol 6000 (PEG); 10, MgCl2·6H2O; 1 and 5 ml dimethyl sulfoxide (DMSO), complete to 100 ml final volume with LB broth then adjust the pH at 6.5 as described by Chung and Miller [25].

Cell suspension was dispensed into sterile Eppendorf tube (200 μl aliquots), which was frozen immediately at −80 °C. Frozen aliquots of the competent cells were allowed to thaw on ice. DNA (ligation mixture) was added to the tube, and then incubated for 20 min on ice. The tube was heat shocked at 42 °C for 60 s then 800 μl of LB medium was added and then incubated at 37 °C for 1 h with shaking. Aliquots (200 μl) were spread on selective LB plates containing ampicillin (amp), X-Gal, and IPTG with a final concentration % 10 mg, 2 mg, and 1 mM, respectively. A master plate for white clones was prepared to test individually clone ability to degrade the cellulose macromolecule.

Screening soil metagenomic clones for expressing cellulase gene
To differentiate the positive cellulase producing clones among soil metagenomic clones (white clones), qualitative detection (plate assay) was carried out. The tested clones were screened for cellulase activity in Nutrient agar (NAamp& IPTG) supplemented with 0.5% of carboxymethylcellulose (CMC). The positive clones characterize by formation of a yellowish halo around the colonies after addition of indicator (0.2% w/v Congo red) followed by washing with 1 M sodium chloride [26].

Enzyme colorimetric assay
The cellulase activity was determined by monitoring the released reducing sugar (measured as glucose) upon enzymatic hydrolysis of CMC substrate, by applying the dinitrosalicylic acid (DNS) method and a standard curve was generated using crystalline glucose powder [27]. Cell pellets of positive clones were sonicated in 2 ml phosphate buffer pH 7. A 0.5 ml of tested pellet lysesate was mixed with 0.5 ml of the CMC-substrate (0.5% w/v) in tris-HCl buffer (50 mM, pH 7.5); the reaction mixture was incubated for 15 min at 50 °C. Afterwards, the reaction was stopped by adding 1 ml of DNS and boiling for 10 min. The released reducing sugar producing color was measured at 540 nm by spectrophotometer (UV, SHI-MADZU); the absorbance was measured against control (without active enzyme). Under standard test conditions, enzyme activity (U) was defined as the amount of enzyme that produced 1 μg of reducing sugar equivalent to glucose per minute. All assays measurements were calculated after subtracting from the individual control which composed of mixture of the enzyme and substrate boiled before being used as a control sample.

Preparation of plasmid-DNA from E. coli recombinant cells harboring insert
Mini-plasmid extraction was carried out via alkali lysis method as defined by Sambrook et al. [24]. Cells (1.5 ml) from overnight culture of the selected clone were collected by centrifugation at 7000 rpm and were sequentially suspended in three solutions (I, II, III) by equal volume; then, using mild shaking after the addition of solution I & II and standing 15 min after solution III. The first (Tris-HCl-pH 7.5 and 100 mM EDTA) was for solubilizing the cell pellet, the second was for lysis (1 M NaOH and 5.3% (w/v) SDS), and the third (60 ml of 5 M K-acetate and 11.5 ml acetic acid w/v%) was for the precipitation of the protein and the high molecular weight DNA. The plasmid containing supernatant was separated after applying centrifugation for 10 min at 13,000 rpm. Finally, the extracted plasmids were precipitated by
isopropanol, were washed with 70% ethanol, and then were suspended in 30 μl water. Polymerase chain reaction (PCR) was applied to amplify the cellulase gene harboured-plasmid by the most active clone(s) using M13 flanking primer (M13F: 5’AGGCCCTGCACCTGAAG3’ and M13R: 5’ TCAGCGCTGGTACC3’ [28]. The PCR was carried out for 30 cycles at 94 °C (denaturation) for 1 min, 55 °C (annealing) for 1 min and 72 °C (extension) for 2 min, followed by 10 min at 72 °C (final extension). After completion, a fraction of the PCR product was examined by using the agarose gel electrophoresis [29], and the remnant mixture was purified by using the QIAquick PCR purification reagent (QIAGEN) according to the manufacturer’s recommendation. Afterwards, PCR product was subjected to automated DNA sequencing using the ABI PRISM model 3730 [30].

Sequence similarity
Basic Local Alignment Tool (BLAST) (www.ncbi.nlm.gov/blast) is an algorithm and is an applied program for comparing the primary biological sequence information such as the nucleotides of DNA and/or ribosome-ribonucleic acid (RNA) sequences and amino-acid sequences of proteins. Thus, it was used to determine the similarity of the received assembled sequence of PCR product-insert (upon using the universal primer of pUC19) with the already submitted sequence in the data base. In addition, multiple sequence alignments were performed by using sequence retrieval system (SwissProt http://hcuge.ch/srs5/). SWISS-PROT is a curated protein sequence data-base which strives to provide a high level of annotation (such as protein-function description, domain structure, post translational modification ... etc.), a minimal level of redundancy, and a high level of integration with other databases.

Expression of recombinant cellulase gene by most active clone under lacZ promoter
This was done by inoculating 50 ml of production LB broth medium (amp & IPTG) dispensed in 250 ml Erlenmeyer flask with 1 ml suspension from freshly prepared overnight pre-culture of the most active clone. A starting pre-culture was grown in 250 ml Erlenmeyer flask with 1 ml suspension from freshly prepared broth medium (amp & IPTG) dispensed in 250 ml Erlenmeyer flask under shaking (200 rpm) at 37 °C. After inoculation of the production medium, flasks were incubated at 37 °C under shaking (200 rpm); growth and cellulase activity were detected along 48 h. Growth was monitored by measuring the optical density (OD600 nm), while the cellulase activity was followed by measuring the color intensity (DNS assay) under the inducing conditions (IPTG, 1 mM). An additional flask was prepared and was run under the same conditions without IPTG to be used as control (non-induced) in the characterization of recombinant cellSNSY through sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and activity staining in the next experiment.

Characterization of the recombinant protein cellSNSY
Protein estimation
In order to estimate the total soluble protein concentration, Lowry method was performed based on pre-prepared standard curve of bovine serum albumin [31].

SDS-PAGE and the activity staining (Zymography)
The recombinant protein samples (induced and non-induced at conc.10–20 μg) were treated with SDS-loading buffer, inactivated for 5 min at 95 °C, suspended on ice, and loaded onto the SDS-PAGE (12 %) according to Laemmli [32]. The gel run for 15 min at 20 mV in the electrode buffer, and then the voltage increased to 60 mV for approximately 3 h. After racing, the gel was stripped from the glass plates, divided into two sections; the first was subjected to stain by Coomassie then de-stained for protein visualization. The second section was incubated for 4 h at 4 °C in the renaturation solution (100 mM Tris–HCl buffer, pH 7.5 containing 0.5% Triton X-100) followed by 2 h at 50 °C in the substrate solution (50 mM Tris–HCl buffer, pH 7.5 containing 0.5% CMC) for cellulase activity detection. Next, the gel was placed in a Petri dish containing Congo-red solution (0.2% w/v) for 10 min at room temperature, and then, it was washed with 1 M sodium chloride. The existence of a faint yellowish halo band indicated that the CMC substrate had been hydrolyzed.

Determination of the optimum temperature
The optimum temperature of the crude recombinant cellSNSY was estimated over different temperatures (ranged 35–80 °C). The reaction was carried out under the initial assay conditions (pH 7.5 and shaking for 15 min).

Determination of the optimum pH
The optimum pH of the crude recombinant cellSNSY was considered upon testing a wide range of pHs (3–10). The experiment was designed by using citrate buffer (pH 3–5); phosphate buffer (6.0–7.0) and tris-HCl (pH 7.5–10) at 50 mM. The reaction assay mixtures were allowed to stand for 15 min under shaking at 45 °C incubation temperature.

Temperature stability
The thermal strength of the recombinant cellSNSY was measured at different temperatures ranging from 25 to 80 °C for (1–2 h), and then the temperature stability of the recombinant cellSNSY protein was checked at −20
°C for up to 9 months without thawing. Furthermore, the stability was tested upon gradual weakly thawing of the tested recombinant protein which was kept in buffer (50 mM tris-HCl buffer pH 7.5) over 12 days at varied period breaks and the residual activity was determined. The reaction assay was carried out under the resulted optimal conditions and was expressed as % compared to untreated enzyme sample.

**pH stability**
The pH stability of the recombinant cellSNSY enzyme was checked at pH 7, 7.5, 8, and 8.5 for up to 9 months. The reaction assay was carried out under the resulted optimal conditions (45 °C, pH 8.5) and was expressed as % compared to untreated enzyme.

**Effect of certain metals, solvents, surfactant, and EDTA on the enzyme activity**
The crude recombinant protein cellSNSY was tested against different cations (MgCl2, CaCl2, ZnCl2, NaCl, FeSO4, MnSO4, and CuSO4), chelating agent (EDTA) at concentrations 1 mM, solvent (DMSO, methanol, ethanol, isopropanol, glycerol), and surfactant (SDS) at concentrations 1% (w/v). The tested enzyme was independently incubated with the tested agent for 15 min; the residual activity was calculated then subtracted from the activity of untreated sample to know the inhibition or activation % of the tested compound.

**Statistical analysis**
All assays were performed in triplex reactions. The results were expressed as means ± standard deviation which was determined by using Microsoft Office Excel 2013.

**Results**

**Metagenomic library construction**
In order to clone positive cellulase clones from Wadi El Natrun, Egyptian soil, a metagenomic technique was applied. Initially, the work started with the isolation of total DNA from sediments collected from these extreme environmental conditions to use in library construction and transform onto E. coli DH5α. The extracted DNA reached to 14,361 ng/μl concentration with a high purity (1.82). Four independent soil-metagenomic libraries were constructed by using BamHI, HindIII, EcoRI, and SalI; 270 transformants clones in total were obtained from these libraries (78, 82, 99, and 11 clones), respectively. White transformant clones were picked and subcultured in a master plate; they were individually screened on LBamp, IPTG-CMC agar plates that were incubated at 37 °C for CMC hydrolysis due to the expression of a heterologous cellulase. Three clones among all the tested clones had shown halo zone by plate assay (Fig. 1A). A quantitative estimation of the cellulase activity for these selected clones (1, 2, and 3) was carried out after 24-h cultivation in LBamp, IPTG-broth medium and was found to give such figures 19, 14, and 8 μ/ml, respectively (Fig. 1B). It is worth to mention that the transformant clone number 1 is derived from BamH1-dependent library, while 2 and 3 from HindIII constructed library. These clones (1, 2, and 3) were picked from master plate and were cultivated in liquid LBamp medium overnight for plasmid isolation. The related recombinant plasmids (pUC-clone 1, 2, and 3) contained an inserted DNA-fragment of about 1.5, 1.2, and 1 Kb as being determined by the PCR-reaction through using M13 flanking universal primer after running (agarose-gel electrophoresis) (Fig. 2). Based on these results, pUC-clone 1 insert was subjected to sequencing, where one consensus partial sequence of 861 bps was obtained after sequence assembly. These nucleotides were translated into amino acids by using BioEdit 7.2 software. The resulted partial sequence composed of 287 amino acids was compared to the data in National Center for Biotechnology Information (NCBI) GenBank via BLAST, where it showed 76% identity to cellulase family glucosyl hydrolase, namely endoglucanase gene of Bacillus amyloliquefaciens (GenBank accession no. AAL99668). The phylogeny of the metagenomic isolated gene designated cellSNSY (287aa) and the closely related genes was analyzed by using the multiple-sequence alignment

![Fig. 1](image-url) Activity screening for isolated recombinant cellulase clones (1, 2 and 3) derived from soil metagenomic. A Well cut method and B DNS method against negative control (buffer).
program (CLUSTALW, Pairwise Alignment), and the results were presented in a phylogenetic tree as shown in Fig. 3.

**Heterologous expression of cellSNSY under lacZ promoter**

After the cloning of cellSNSY gene (namely endoglucanase), its expression was monitored under lacZ-promoter using IPTG (1 mM) (Fig. 4). It was noticed that the growth and the cellulase activity of the recombinant cellSNSY were progressively increasing by time elongation up to 27 h (the end of exponential growth phase) with no noticeable increase either in growth or activity.

**Characterization of the recombinant cellSNSY**

Quick analysis of recombinant proteins derived from cellSNSY (cultivated under induced and non-induced conditions) was performed through SDS-PAGE and activity stain. It was recognized in Fig. 5 the appearance of an active band at molecular weight ~59 kD by inducing sample.
Effect of temperature

As described in the “Methods” sections, different temperatures (35–85 °C) were tested to determine the optimal value for the recombinant cellSNSY. The data plotted in Fig. 6 explained that the optimal activity had appeared at 45 °C. On the other hand, the recombinant cellSNSY showed a complete stability at temperatures (20, 30, 35 °C); (40, 45 °C); and (50, 55 °C) for 4 h, 2 h, and 50 min (data not shown), respectively. Whereas enzyme exposure to higher temperatures (55, 60, and 70 °C) caused noticeable drop in the thermal stability of the recombinant cellSNSY which were approximately equal 14, 34, 42, and 49% (data not shown) after 15-min exposure, respectively. This means the half-life of crude recombinant cellSNSY was 15 min at 70 °C. On the other hand, stability of the recombinant cellSNSY was tested upon being freezed at −20 °C (without thawing condition) throughout 20 weeks with 2 weeks intervals (Fig. 7). Data in this figure indicated that the recombinant cellSNSY was highly stable under such freezing condition and 30% of enzyme activity was lost after 8 weeks. However, time extension caused continuous drop in the retained activity and 50% loss had resulted after 12 weeks as shown in Fig. 7. In addition, the stability was checked upon gradual weakly thawing (up to 10 times freezing/thawing) of the recombinant protein (cellSNSY) samples along 20 days where 94% loss in the enzyme activity of cellSNSY was noticed after ten times (data not shown).
Effect of pH

The crude recombinant cellSNSY enzyme activity rate was studied as a function of the pH ranging from 3 to 10. The results presented in Fig. 8 explained that the recombinant cellSNSY had worked in an alkaline condition giving its optimal at pH 8.5.

The pH stability of the recombinant cellSNSY was monitored in an alkaline condition (pH 7.5, 8.0, and 9.0) along 240 min (4 h) at room temperature (data not shown) where it showed complete pH stability at these tested pH. Alongside, the cellSNSY recombinant stability was tested upon the preservation at different pHs (7.5, 8.0, and 8.5) and under cooling (−20 °C) for 8 weeks and was compared to untreated enzyme. The data illustrated in Fig. 9 indicated that the tested cellSNSY withstood the preservation under alkaline pH 8.5 > 8.0 > 7.5. The highest loss in activity % (50, 35, and 15) occurred after 8 h of preservation at pH 7.5, 8.0, and 8.5, respectively.
Effect of different compounds
Metal ions can be binder to proteins and to other enzyme-related molecules to form complexes. The effect of different cations, surfactants, solvents, and metal chelators were tested and the response of *cellSNSY* toward the tested substances was reported and was shown in Table 1. The outcomes indicated that the presence of MgCl₂, CaCl₂, NaCl, glycerol, DMSO did not decrease the activity of *cellSNSY* at all. ZnCl₂, ethanol, and isopropanol showed very limited decrease in activity (ranged 1–2%). Moreover, a noticeable decrease in % of the residual activity around (30, 15, 13, and 13) was caused by treating the investigated enzyme with the following heavy metals: CuSO₄, MnSO₄, FeSO₄, and surfactant (SDS), respectively as shown in Table 1.
Table 1 Effect of some metal ions, SDS, solvents, and inhibitors on the activity of recombinant cellSNSY enzyme activity

| Tested                      | Different metal ions | Surfactants and solvents | Metal chelate |
|-----------------------------|----------------------|--------------------------|---------------|
| Cu²⁺                       | 71.32 ± 0.5          | 87.7 ± 0.8               | 95.5 ± 2.2    |
| S.1.1.1. Ca⁺                | 101.42 ± 1.2         | 94.1 ± 1.2               | 100 ± 0.0     |
| S.1.1.2. Na⁺                | 100.54 ± 1.3         | 99.7 ± 0.82              | 100 ± 0.0     |
| S.1.1.3. Mg⁺²               | 102.1 ± 0.99         | 100.6 ± 0.8              | 100 ± 0.0     |
| S.1.1.4. Mn⁺²               | 85. ± 0.8            | 98.2 ± 1.4               |               |
| S.1.1.5. Fe⁺²               | 87.5 ± 1.34          | 100.6 ± 0.8              |               |
| S.1.1.6. Zn⁺²               | 99.55 ± 2.2          |                          |               |
| S.1.1.7. Control (untreated enzyme) | 100 ± 0.0           |                          |               |

Discussion

Cellulose is considered one of the most abundant agriculture wastes. It constitutes the major structural components of the cell walls of higher and lower plants. Huge amounts are produced annually, but its accumulation is problematic. This study had been directed to look for a new cellulase enzyme with specific properties to be useful in many applicable fields. In order to reach this target, the study aimed for cloning a positive cellulase recombinant(s) from Wadi El Natrun, Egyptian, soil by implementing a metagenomic technique. Initially, the work started with the isolation of total DNA from soils which were collected from the extremely saline and alkaline lakes of the Wadi El Natrun to be used in library construction and to be transformed onto E. coli DH5α. Soda lakes are found all over the world and are classified as a highly productive environment, though, being extreme. Such locales are considered good supplies for novel species of microbes; the extremophilic nature of these microorganisms will make them a potential source for enzymes and metabolites with industrial uses [33]. Most of the microbes survived in extreme environment and are difficult to be cultured in the laboratory. Therefore, metagenomic technique (culture-independent method) facilitates the isolation of novel enzymes (genes) from the total microbial population including the unculturable one by using material extracted directly from environmental samples [34].

Recently, a wide variety of hydrolases have been cloned from environmental DNA via applying metagenomic technique [35–38].

A successful metagenomic creation mainly depends on DNA quality and concentration where the low DNA concentration, difficulty in extraction, and cloning are major obstacles. In this study, soil DNA collected from saline and alkaline lakes of the Wadi El Natrun was successfully extracted (conc. 14,361 ng/μl) with a good purity (1.82). Afterwards, soil metagenomic library was constructed by using the restriction enzymes (BamHI, HindIII, EcoRI, and SalI) and 270 transformants clones that were obtained as based on blue/white screening in the presence of X-gal and IPTG. The obtained numbers of transformants (270 clones) indicated a low cloning efficiency result which needed to be improved. This might be related to the presence of some inhibitors like humic acid in environmental samples. Humic acid was found as a coextracted; its existence caused an inhibition of nucleases restriction enzyme and a limitation in transformation processes [39].

Cellulolytic activities of liberated clones were monitored; three active clones were recognized by a yellow halo zone of hydrolysis (plate assay). The most active clone (clone 1) with the maximum cellulase activity was further identified as a novel cellulase gene via analysis of the nucleotide and amino acid sequence. A partial amino acid sequence of cellSNSY (287aa) showed 76% identity to cellulase family glucosylhydrolase, namely endoglucanase gene of Bacillus amyloliqufaciens (GenBank accession no. AAL99668). In general, cellulase is not a single enzyme (a group composed of endoglucanase and exoglucanases) that are including cellobiohydrolyases and β-glucosidase. These classes work in synergistic way in order to completely hydrolyze the cellulose molecule [38]. As far as it is concerned here, this is the first report about the isolation of endoglucanase related cellulase gene through metagenomic library from Wadi El Natrun by functional screening. The cellSNSY gene derived from metagenomic library was cloned and was expressed under lacZ promoter in E. coli DH5 using IPTG (1 mM) where its crude protein was characterized. The expressed cellSNSY showed an active band with a molecular mass (~59 kDa) through zymography (in situ detection of cellulolytic activity). This obtained molecular weight was nearer the cellulase from a thermophilic Paenibacillus barcinonensis 58.6 kDa [40], and that from Bacillus mycoides (62 kDa) [41]. Consequently, the crude recombinant cellSNSY was subjected to intensive characterization to explore its uniqueness in traits which further nominated it for some specific applications. The maximum activity of cellSNSY was recorded at 45 °C. This result agreed with the results obtained by [42] who
found the optimum temperature for Aspergillus niger MK543209 cellulase as being 45 °C. Li et al. [43] reported that most thermophiles were having optimum temperature ranging between 65 and 70 °C. Therefore, it was recognized that about 45% of cellulose degradation have occurred due to the action of bacterial consortia at 37 °C [44]. Patel et al. reported the novel cellulase named Cel5M from rumen metagenome which showed maximum activity at 40 °C [45]. Thermal stability of enzymes is considered a very important feature for industrial applications. By testing cellSNSY, it was found to be completely stable at tested temperatures 25–55 °C for about 1 h. However, it lost ~50% of its activity at 70 °C after 15 min of exposure. Patel et al. [45] recorded the novel recombinant Cel-5M retained 65% for its activity by measuring the thermal stability between 30 and 70 °C. Ogonda et al. [46] demonstrated that the cellulase enzyme of Bacillus sp. had retained approximately 99 and 40% of activity at 60 °C and 80 °C, respectively. Islam et al. [47] stated that about 68% of the activity had been retained after heating the crude cellulase enzyme solution from Bacillus sp. at 50 °C for 30 min. These results were in contrast with those reported by Li et al. [48] who stated that cellulose produced by Bacillus tequilensis strain GY1H001 from Angelica can remain active even it was heated at 100 °C for 30 min. It is worth mentioning that cellulases are characterized by a high thermal stability that designates them for rendering for sustainable agriculture, cellulose-based research, and industrial processes.

Likewise, cellSNSY, temperature stability was monitored also under freezing and the enzyme showed a complete stability up to 8 weeks. This means that the enzyme is able to survive for long term preservation under freezing conditions.

As it was previously reported, the cellSNSY under investigation had indicated preferable tolerance to act in an alkaline condition where the optimum was recorded at pH 8 by showing complete pH stability under alkaline conditions (pH 7.5–8.5). This finding is in contrast with the recombinants designated Cel-5M and Cel14b22 which showed maximum activities at pH 6.0 and 6.0–7.0 [45, 49], respectively. Also, optimum pH was found to be 6.5–7.5 for the cellulose-degrading bacteria which were isolated from Saliva Breed of Buffalo [50]. To add more, a metagenomic-derived Cel5M had retained more than 80% activity between 4 and 7 pH as described by Patel et al. [45]. Some researchers stated that cellulases are generally stable over a wide range of pH from 5.0 to 10.0 [51, 52], especially thermophiles are usually having an optimum pH that ranged (pH 6–10) [43]. Gong et al. [49] found that the recombinant metagenomic cellulases have a broad stability that are ranging from pH 4.0 to 10.0.

It is worth to mention that the increasing or the decreasing the pH changes the ionic state of ionizing side chains of protein molecule, disrupts ion pairs, breaks hydrogen bond, and consequently denatures the protein [53]. In contrast to results given here in this study, the previous findings reported that acid cellulase from Aspergillus niger showed the highest activity at pH 2.5 [54]. Maleki et al. [55] have been trying to develop a cocktail (PersiCell I&2) of novel thermostable cellulases with high hydrolytic ability and stability as the first PersiCell I works optimally at pH 8.0 and the PersiCel2 optimally active at the pH of 5.

Metal ions can form complexes in association with proteins and other molecules that are related to enzymes. They may act as donors or acceptors of the electron as structural regulators [56].

The effect of different cations, surfactants, and EDTA on the crude recombinant enzyme (cellSNSY) activity was tested. The results showed that the presence of cations such as Mg$^{2+}$, Ca$^{2+}$, Na$^+$, and Zn$^{2+}$ did not decrease the activity. Fe$^{2+}$, Mn$^{2+}$, and Cu$^{2+}$ caused a decrease in the cellSNSY enzyme activity. These results agreed with the results obtained by many researchers [57–59], who reported that both Ca$^{2+}$ and Na$^+$ cations exhibited stimulatory effects on the enzyme activity in all tested concentrations. The results of the under investigations are in agreement with Asha et al. [40] who reported for the activity of the purified cellulase from Paenibacillus barcinonensis which is stimulated in the presence of metal ions such as Mg$^{2+}$, Mn$^{2+}$, and Co$^{2+}$. Also, Chai et al. [22] found that the cellulase activity was increased by Mn$^{2+}$, Ca$^{2+}$, Zn$^{2+}$, and Mg$^{2+}$ at 1 mM. In contrast to ones' results, Akintola et al. [57] reported that Mg$^{2+}$ inhibited the activity of cellulase from Enterobacter cloacae IP8 at concentration 4 mM to 15 mM; it stimulated its activity at concentrations (ranged 20–200 mM). Gong et al. [49] noticed that the recombinant Cel14b22 was considerably enhanced by Mn$^{2+}$, but intensely reduced by Fe$^{3+}$ or Cu$^{2+}$.

By testing EDTA (1 mM), the studied recombinant cellSNSY lost 5% of its activity, while Akintola et al. [57] reported that EDTA at concentrations above 4 mM inhibited the activity of the crude cellulase from the bacterium (Enterobacter cloacae IP8). Similarly, EDTA caused the inhibition of cellulase which was produced by Acetobacter xylinum Ku-1 [60]. EDTA as a metal chelating agent probably was acted by the inactivating of the cellulase either by removing metal ions from the enzyme through the formation of coordinating complex, or by binding them inside the enzyme as a ligand as had been noted by Schmid [61].

Additionally the recombinant cellSNSY showed a resistance to the tested solvents like DMSO, alcohols, and
glycerol, although the surfactants (SDS) had been caused around 15% drop in the activity. By the same token, SDS surfactant caused around 34% of inhibition in cellulase activity from *Acinetobacter junii* GAC 16.2 [62].

**Conclusions**

Wadi El Natrun microorganisms have been considered a pronounced source for discovery of new natural products and metabolites. Thus, a functional metagenomic route was followed for cloning a novel cellulose gene from environmental soil samples collected from Wadi El Natrun. Samples existed under harsh conditions. Saline and alkaline were selected to prospectively find an enzyme that can be able to withstand the harsh conditions. A successful soil-metagenomic library was developed, but low transformation efficiency was recognized. Activity screening for transformants had discriminated many active clones regarding cellulose hydrolysis by plate assay and the monitoring of the liberated reducing sugars (DNS colorimetric assay method). Moreover, the most active clone designated cellSNSY had shown an active protein band at molecular mass ~ 59 kD through SDS-PAGE and activity staining. Molecular characterization for cellSNSY had shown 76% identity to cellulase family glucosylhydrolase, namely endoglucanase. Characterization of crude recombinant protein explained that the cellSNSY works optimally at 45 °C and pH 8.5. Its half-life is 15 min at 70 °C. It showed great stability under alkaline conditions, and it is suitable for long-term preservation under freezing conditions. It showed complete stability toward the tested alcohols, DMSO, glycerol, and heavy metals like Zn²⁺. The highest inactivation (~30%) caused by Cu²⁺ followed by Mn²⁺ (~15%) and Fe⁺² (~13%). A 1% surfactant (SDS) postponed the activity by 12%. The overall features and properties of the studied cellSNSY made it suitable for many future applications in industries; like textile, paper, and cellulose dependent researches.

**Abbreviations**

CMC: Carboxymethylcellulose; DMSO: Dimethyl sulfoxide; EDTA: Ethylene diamine tetra acetic acid; SDS-PAGE: Sodium dodecyl sulfate–polyacrylamide gel electrophoresis; SDS: Sodium dodecyl sulfate; amp: Ampicillin; X-gal: 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside; IPTG: Isopropyl β-D-1-thiogalactopyranoside; DNS: Dinitorosalicylic acid; PEG: Polyethylene glycol; RNA: Ribosome-ribonucleic acid; DNA: Deoxyribonucleic acid; PUC: Plasmid cloning vector; PCR: Polymerase chain reaction; BLAST: Basic Local Alignment Tool; NCBI: National Center for Biotechnology Information; MEGA: Molecular evolutionary genetics analysis; aa: Amino acids; OD: Optical density; TSS: Transformation storage solution; E. coli: *Escherichia coli*; U: Units; w/v: Weight/volume; h: Hour(s); min: Minute(s); sec: Second(s); rpm: Rotation per minute; bp: Base pair; kDa: Kilo Dalton; Kb: Kilobase pair; LB: Luria-Bertani; NA: Nutrient agar.

**Acknowledgements**

The authors are extremely grateful to the City of Scientific Research and Technological Applications (SRTA-City), Alexandria, Egypt, for providing all facilities to complete this work.

**Authors' contributions**

SMA performed the experimental part of the work and performed the analysis part. NAS designed the experiments. SAA wrote the main manuscript text. YRA revised the manuscript. All authors read and approved the manuscript.

**Funding**

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

**Availability of data and materials**

All data generated or analysed during this study are included in this published article.

**Declarations**

**Ethics approval and consent to participate**

This article does not contain any studies involving animals or human participants performed by any of the authors.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare no competing interests.

**Author details**

1. Nucleic Acid Research Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), City of Scientific Research and Technological Applications, Alexandria, Egypt. 2. Present address: City of Scientific Research and Technological Applications (SRTA-City),, New Burg El-Arab City, Universities and Research Institutes Zone, Alexandria Post 21934, Egypt. 3. Bioprocess Development Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), City of Scientific Research and Technological Applications (SRTA-City), Alexandria, Egypt.

**Received:** 10 June 2021  **Accepted:** 30 January 2022  **Published online:** 08 February 2022

**References**

1. Zhao X, Liu L, Deng Z, Liu S, Yun J, Xiao X, Li H (2021) Screening, cloning, enzymatic properties of a novel thermostable cellulase enzyme, and its potential application on water hyacinth utilization. Int Microbiology 24:337–349
2. Saddiqi H, Ollaei E, Honarkar H, Jin J, Geonzon LC, Bacabac RG, Klein-Nulend J (2021) Cellulose and its derivatives: towards biomedical applications. Cellulose 28:1893–1931
3. Hussain AA, Abdal-Salam MS, Abo-Ghaliia HH, Hegazy WK, Hafez SS (2017) Optimization and molecular identification of novel cellulose degrading bacteria isolated from Egyptian environmental soil. J Gene Eng Biotechnol 15:77–85
4. Husseen HA, Darwesh OM, Mekki BB (2019) Environmentally friendly nano-selenium to improve antioxidant system and growth of groundnut cultivars under sandy soil conditions. Biocat Agric Biotechnol 18:101080
5. Ates B, Koytepe S, Ulu A, Gurses C, Thakur VK (2020) Chemistry, structures, and advanced applications of nanocomposites from biorenewable resources. Chem Rev 120:9304–9362
6. Tarche D, Thakur VK, Bouherroub R (2020) Cellulose nanocrystals/graphene hybrids—a promising new class of materials for advanced applications. Nanomaterials 10:1523
7. Sukumaran RK, Singhania RR, Pandey A (2005) Microbial cellulases-production, applications and challenges. J Sci Indus Res 64:832–844
8. Juturu V, Wu JC (2014) Microbial cellulases: engineering, production and applications. Ren Sustain Energy Rev 33:188–203
9. Hasanin MS, Darwesh OM, Matter IA, El-Sayed H (2019) Isolation and characterization of non-cellulolytic *Aspergillus flavus* EGYPTAS exhibiting selective ligninolytic potential. Biocat Agric Biotechnol 17:160–167
10. Acharya A, Joshi D, Shrestha K, Bhatta D (2012) Isolation and screening of thermophilic cellulolytic bacteria from compost piles. Sci World 1043–46
11. Kuhad RC, Gupta R, Singh A (2011) Microbial cellulases and their industrial applications. Enzyme Research
12. Binder JB, Raines RT (2010) Fermentable sugars by chemical hydrolysis of biomass. Proc Natl Acad Sci USA 107:4516–4521
13. Sunung N, Ray S, Bose S, Rai V (2013) A broader view: microbial enzymes and their relevance in industries, medicine, and beyond. BioMed Research International
14. Adrio JL, Demain AL (2014) microbial enzymes: tools for biotechnological processes. Biomol 4:117–139
15. Hussein HA, Darwesh OM, Mekki BB, El-Hallouty SM (2019) Evaluation of cytotoxicity, biochemical profile and yield components of groundnut plants treated with nano selenium. Biotechnol Rep. 24:e00377
16. Marrez DA, Abdelhamid AE, Darwesh OM (2019) Eco-friendly cellulose acetate green synthesized silver nano-composite as antibacterial packaging system for food safety. Food Pack Shelf Life 20:100302
17. Moussa TA, Tharwat N (2007) Optimization of cellulase and β-glucosidase induction by sugar beet pathogen Sclerotium rolfsii. Afr J Biotechnol 6(8):1048–1054
18. Darwesh OM, Sultan YY, Seif MM, Marrez DA (2018) Bio-evaluation of crustacean and fungal nano-chitosan for applying as food ingredient. Toxicol Rep 5:348–356
19. Poli A, Finore I, Romano I, Gioiello A, Lama L, Nicolaus B (2011) Microbial diversity in extreme marine habitats and their biomolecules. Microorganisms 3(5):23–30
20. Tiwari R, Nain L, Labrou NE, Shukla P (2018) Bioprospcting of functional cellulases from metagenome for second generation biofuel production: a review. Crit Rev Microbiol 44:244–257
21. FAdi P, Budiharjo A, Kusumaningrum HP, Wijanarka W, Suprihadi A, Ali et al. Journal of Genetic Engineering and Biotechnology 2022:20:20
22. Chung CT, Miller RH (1993) Preparation and storage of competent cells. In: Methods Enzymol:621–627
23. Pandey M, Singhal B (2021) Metagenomics: adding new dimensions in bioeconomy. Biomass Conversion and Bioenergy 1–20
24. Sambrook J, Fritsch EF (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, New York, p 1546
25. Chung CT, Miller RH (1993) Preparation and storage of competent Escherichia coli cells. In: Methods Enzymol 621–627
26. Kluepfel D (1986) Screening of procaryotes for cellulose- and hemicellulose degrading enzymes. Methods Enzymol 160:180–186
27. Miller GL, Blum R, Glennon WE, Burton AL (1960) Measurement of carboxymethylcellulose activity. Anal Biochem 2.127–132
28. Soliman NA, Knoll M, Abdel-Fattah YR, Schmid RD, Lange S (2007) Molecular cloning and characterization of thermostable esterase and lipase from Geobacillus thermoleovorans Tn isolated from desert soil in Egypt. Process Biochem 42:1090–1100
29. Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K (1999) Short protocols in molecular biology. Wiley, NY
30. Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain terminating inhibitors. Proc Natl Acad Sci 74:5463–5467
31. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193(1):265–275
32. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685
33. Margesin R, Schinner F (2001) Potential of halotolerant and halophilic microorganisms for biotechnology. Extremophiles 5:73–83
34. Rees HC, Grant S, Jones BE, Grant WD, Heaphy S (2003a) Detecting cellulase and esterase activities encoded by novel genes present in environmental DNA libraries. Extremophiles 7:415–421
35. Peng Q, Zhang X, Shang M, Wang X, Wang G, Li B, Guan G, Li Y, Wang Y (2011) A novel esterase gene cloned from a metagenomic library from neritic sediments of the South China Sea. Microbial Cell Factories 10(9).
36. Chuzel L, Ganatra MB, Rapp XE, Hennissat B, Taron CH (2018) Functional metagenomics identifies an exo-α-cellobiohydrolase with an inverting catalytic mechanism that defines a new glycoside hydrolase family (GH106). J. Biol Chem 293(47):18138–18150
37. Escuder-Rodriguez J, DeCastro ME, Cerdán ME, Rodríguez-Belmonte E, Becerra M, González-Sisco MI (2018) Cellulases from thermophiles found by metagenomics. Microorganisms 6:1–26
38. Pabballi N, Veldarandi A, Tavanna T, Gupta S, Raj RS, Gandam PK, Baadhe RR (2021) Role of metagenomics in prospecting novel endoglucanases, accentuating functional metagenomics approach in second-generation biofuel production: a review. Biomass Convers Biorefin 1–28
39. Tebbe CC, Vahjen W (1993) Interference of humic acids and DNA extracted directly from soil in detection and transformation of recombinant DNA from bacteria and yeast. Appl Environ Microbiol 59(8):2657–2665
40. Asha BM, Ravathi M, Yadav A, Sakhthivel N (2012) Purification and characterization of a thermostable cellulase from a novel cellulytic strain, Paenibacillus barcinonensis. J. Microbiol Biotechnol 22:1501–1509
41. Balasubramanian N, Toubardo D, Teseima E, Simons N (2012) Purification and biochemical characterization of a novel thermo-stable carboxymethyl cellulase from azorean isolate Bacillus mycoderis 5122. Appl Biochem Biotechnol 168:2191–2206
42. Darwesh OM, El-Maraghy SH, Abdel-Rahman HM, Zaghloul RA (2020) Improvement of paper wastes conversion to bioethanol using novel cellulose degrading fungal isolate. Fuel 262:116518
43. Li Y, Tscheslikinski TJ, Engle NL, Hamilton CY, Rodriguez M, Liao JC, Schadt CW, Guiss AM, Yang Y, Graham DE (2012) Combined inactivation of the Clostridium cellulolyticum lactate and malate dehydrogenase genes substantially increases ethanol yield from cellulose and switchgrass fermentations. Biotechnol Biofuels 5:2
44. Karthika A, Seenivasagan R, Kasimani R, Babalola OO, Vasanthy M (2020) Cellulolytic bacteria isolation, screening and optimization of enzyme production from vermicompost of paper cup waste. Waste Manage 116:58–65
45. Patel M, Patel Hiral M, Dave S (2020) Determination of bioethanol production potential from lignocellulosic biomass using novel Cel-Sri isolated from cow rumen metagenome. Int J Biolum 153:1099–1106
46. Ogonda LA, Muge EK, Wamalwa BM, Mulua FJ, Teller C (2020) Characterization of crude cellulases from a Bacillus sp. isolated from Lake Bogoria. Kenya. Research square. 1–27. https://doi.org/10.21037/rso.3.r3-41635/v1
47. Islam M, Sarkar PK, Mohiuddin MM, Suzauddina M (2019) Optimization of fermentation condition for cellulase enzyme production from Bacillus sp. Malaysian Journal of Halal Research 2(219–24)
48. Li H, Guan Y, Dong Y, Zhao L, Rong S, Chen W, Li M, Xu H, Gao X, Chen R, Li L, Xu Z (2018) Isolation and evaluation of endophytic Bacillus tequilensis GYEL001 with potential application for biological control of Magnaporthe oryzae. PLOS One:1–18
49. Gong X, Gruninger RJ, Qi M, Paterson L, Forster RJ, Teather RM, McAllister (2020) Characterization of a thermophilic cellulase from a novel cellulolytic strain, Bacillus steinheili. J. Microbiol Biotechnol 30(1):566
50. Shinde A, Shaike F, Pisal K, Patil P (2020) Isolation and characterization of cellulose degrading bacteria from saliva of Jaffnabadi/Breed of Buffalo. Int J Livest Res 10(9):96–104
51. Kim SJ, Lee CM, Kim MY, Yeo YS, Yoon SH, Kang HC, Koo BS (2007) Screening and characterization of an enzyme with betaglucosidase activity from environmental DNA. J. Microbiol Biotechnol 17:905–912
52. Tahir SR, Baksh A, Rao AQ, Naz M, Saleem M (2019) Isolation, purification and characterization of extracellular β-glucosidase from Bacillus sp. Adv. Environ. Biol 3(3):269–277
53. Rawin JD (1989) Biochemistry, 1st edn. Neil Patterson Publishers, Burlingam
54. Ikeda R, Yamamoto T, Funatsu M (1973) Chemical and enzymatic properties of acid cellulase produced by Aspergillus niger. Agr Biolum 37(5):1196–1175
55. Maleki M, Shahrahki MF, Kavousi K, Ariaenejad S, Salekdeh G, H. (2020) A novel thermostable cellulase cocktail enhances lignocellulosic bioconversion and biorefining in a broad range of pH. Int J Biolum 2020(15):349–360
56. Riondian JF (1977) The role of metals in enzyme activity. Ann Clin Lab Sci 7:119–129
57. Akintola AI, Oyedeji O, Adewale IO, Bakare MK (2019) Production and physicochemical properties of thermostable, crude cellulase from Enterobacter cloacae IP8 isolated from plant leaf litters of Lagerstroemia indica. Linn. J. Microbiol Biotechnol Food Sci 8(4):989–994.
58. Femi-Ola TO, Olowe BM (2011) Characterization of alpha amylase from *Bacillus substilis* BS isolated from Amistermes evuncifer Silvestri. Res J Microbiol 6(2):140–146

59. Gaur R, Tiwari S (2015) Isolation, production, purification and characterization of an organic-solvent-thermostable alkaliophilic cellulase from *Bacillus vallismortis* RG-07. BMC Biotechnol 15(19):1–12

60. Oikawa T, Takagi M, Ameyawa M (1994) Detection of carboxymethyl cellulase activity in *Acetobacter xylinum* Ku-1. Biosci Biotechnol Biochem 58:2102–2103

61. Schmid FX (1997) Optical spectroscopy to characterize protein conformation and conformational changes. In: Creighton TE (ed) Protein structure: a practical approach, vol 360, 2nd edn. TRL Press, Oxford, New York and Tokyo

62. Banerjee S, Maiti TK, Roy RN (2020) Production, purification, and characterization of cellulase from *Acinetobacter junii* GAC 16.2, a novel cellulolytic gut isolate of *Gryllotalpa africana*, and its effects on cotton fiber and sawdust. Ann Microbiol 70(28):2–16

**Publisher’s Note**

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.