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Determination of Cellulase Enzyme Produced by *Bacillus cereus* DU-1 Isolated from Soil, and Its Effects on Cotton Fiber

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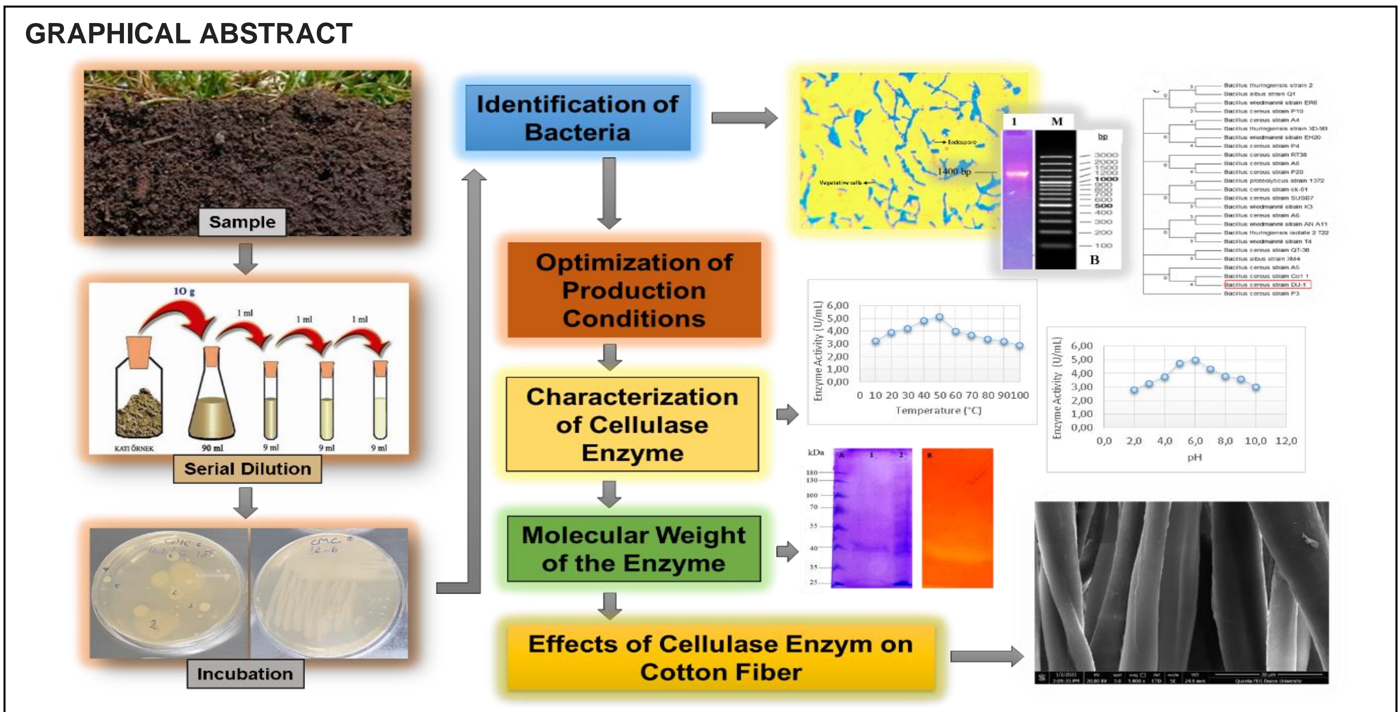
HIGHLIGHTS

- A report showing was identified as *Bacillus cereus* DU-1 which is a cellulase-producing bacterium.
- Optimum production conditions for *Bacillus cereus* DU-1 are 37°C, pH 7 and 24 h, respectively.
- The Vmax value of the enzyme was 3.18 U/mL and the Km value was 0.0019 mM.
- The performance of cellulase enzyme on denim fabrics was evaluated by electron microscopy.

Abstract: A Cellulase enzyme is the enzyme synthesized by some bacterial and fungal species and breaks down the ß-1,4 glycoside bonds of cellulose. Cellulase enzyme has a wide range of uses such as food, paper, textile industry, animal feed and detergent production. In this study, which was designed to add a new cellulase enzyme with such a wide industrial use, cellulotic bacterium isolation, identification, partial purification, characterization and application on denim fabric of the cellulase enzyme synthesized by the identified bacterium was performed. Firstly, bacterium that can produce cellulase enzyme was isolated from soil samples containing hazelnut wastes. The enzyme-producing isolate was identified as *Bacillus cereus* strain DU-1 by morphological, biochemical and molecular techniques. The optimum conditions for enzyme production by *B. cereus* DU-1 was determined as pH 7.0, at 37°C and 24h. However, the activity of the enzyme (5.16 U/mL) was found to be optimum at 50°C and pH 6.0. The Vmax value of the enzyme was 3.18 U/mL and the Km value was 0.0019 mM. The enzyme was partially purified by ammonium sulfate precipitation. The molecular size of the partially purified enzyme was determined as approximately 40 kDa by SDS-PAGE and Zymogram analysis. Finally, enzyme was applied on the denim fabric and when the fabrics were viewed under the electron microscope, it was seen that they were suitable for textile use.

Keywords: *Bacillus cereus*; Cellulase; Characterization; Enzyme kinetics; Purification.



INTRODUCTION

Enzymes that perform important metabolic functions in cells; they are generally protein-structured molecules synthesized by plants, animals and microorganisms. Enzymes can regulate the rate and specificity of many reactions in the cell without changing. Many metabolic reactions are controlled and accelerated by enzymes [1]. Most of the enzymes used in industry are obtained from microorganisms, but a small part is obtained from plants and animals. Enzymes produced by microorganisms are preferred in the industry due to the fact that their catalytic activities are higher than animal and vegetable sources, they form fewer by-products, they are more durable, and they can be obtained cheaply and in high purity [2]. It is observed that approximately 2,500 different enzymes have been discovered and 80% of all enzymes used in the industrial sector are composed of enzymes of microbial origin [3]. It is known that protease, amylase and cellulase enzymes are needed in industrial applications, respectively. However, among the enzymes used industrially, cellulase enzymes that hydrolyze cellulose are seen to be an important biotechnological enzyme in the industrial field after amylases and proteases.

Cellulose is the main component of lignocellulosic biomass that is hydrolyzed to glucose by cellulase enzymes. Lignocellulosic biomass is abundant in soil in the form of agricultural and forest residues. This biomass is an energy source for various bacteria, fungi and insect species, as well as creating a habitat for them [4].

Cellulase enzymes are naturally needed to maintain life in order to hydrolyze cellulose, which is known as the most abundant natural organic resource on earth. At the same time, cellulases are important to reduce high costs and create more efficient processes in all industrial production, including cellulotic products [5]. Microbial cellulases have been an important research topic for many industrial research groups and academia, as they have many industrial uses such as textile, paper, food, detergent, feed, biofuel production, fruit juice and agriculture [6]. Cellulase enzyme, which has a wide application area, has become highly preferred in the textile industry, in enzymatic processes such as fabric softness, good performance, biopolishing and obtaining the desired appearance [7–9]. Cellulase enzymes that hydrolyze cellulose are used to smooth the fabric surface, reduce pilling tendency, biopolishing and give denim fabrics an aged look [7,10]. Biopolishing is a process performed by removing cellulose fibrils from the outer surface of the fiber to reduce pilling. This is accomplished through the hydrolysis of β-1,4 glycosidic bonds. Cellulase enzymes are frequently used for the biopolishing of cellulosic fabrics such as cotton [7,9]. With the application of cellulase, a smoother and softer structure is formed in the surface morphology. Changes in surface morphology can be visualized by scanning electron microscopy (SEM) for all fibers [11,12].

It has been revealed by many studies that cellulase enzymes have a wide range of applications from the food industry to the agricultural industry, from the textile industry to the paper industry, from the

pharmaceutical industry to the chemical industry. This study was designed to add a new one to cellulase enzymes that have such a wide sectoral use. For this purpose, in this study, cellulase enzyme producing bacteria was isolated from the soil containing hazelnut waste, and then the enzyme produced was characterized and applied on denim fabric.

MATERIAL AND METHODS

Isolation of Bacteria

For the isolation of bacteria producing cellulase enzyme, firstly, samples were taken from the soil found by the hazelnut waste (Düzce Province, Akçakoca District, Yenice Village; 41°01´07.4´´N 31°01´04.1´´E) and transported to the laboratory with sterile tubes. 1 g of soil was taken from each sample and serial dilution was made with 9 mL sterile dH_2O. Samples of the 10^{-1}, 10^{-2} and 10^{-3} dilutions were spread on Nutrient Agar (NA) and incubated for 16-18 h at 37°C. At the end of the period, pure bacterial isolates with different morphological appearances were obtained.

Qualitative Enzyme Activity

The bacterial isolates were incubated for 16-18 h at 37°C by spot seeding on enzyme production medium (CMC Agar; Tryptone; 10 g/L, Yeast Extract; 5 g/L, NaCl; 5 g/L, Agar; 15 g/L, CMC (Carboxymethylcellulose); 5 g/L). As a result of the incubation, the petri dish found by the bacteria developed as a single colony was stained with 0.1% Congo red and then incubated with the dye for 5 minutes. Then, the dye was removed by adding 1M NaCl solution to the petri dishes [13]. Cellulase activity was determined according to the yellow zone formation around the bacterial colony.

Identification of Bacteria

Morphological and Biochemical Identification

The gram characteristic of cellulase producing bacteria was determined with 3% KOH [14]. Then, the cellulase producing isolate was stained with simple staining technique and cell morphology was determined by light microscopy. In addition, it was determined whether the isolate had the ability to produce crystals by crystal dyeing [15]. The biochemical properties of cellulase producing bacteria were analyzed with the VITEK 2 system.

Molecular identification and Phylogenetic analysis

For molecular identification of the strains, first, genomic DNA was extracted [16]. The polymerase chain reaction (PCR) amplification of the 16S rDNA genes using genomic DNA was then performed using oligonucleotide primers. The reaction was carried out in a volume of 50 µL for UNI16S-L: 5’- ATT CTA GAG TTT GAT CAT GGC TTC A-3’ UNI16S-R: 5’-ATG GTA CCG TGT GAC GGG CGG TGT TGT A-3’. The PCR content consisted of 2.5 units Taq DNA polymerase (Thermo), 5 µL 5×PCR buffer, 3 µl $MgCl_2$ (1 mM), 1.5 µL forward primer (100 mM), 1.5 µL reverse primer (100 mM), 1 µL dNTP (100 mM), and 1 µL genomic DNA, and sterile ddH20. The PCR conditions consisted of 36 cycles carried out after 2 min of denaturation at 95°C, at 94°C for 1 min, at 50°C for 1 min, at 72°C for 2 min and finally, at 72°C for 5 min. PCR samples were sent to Letgen (Turkey) for subsequent identification. After the base sequence of the 16S rRNA gene obtained as a result of the sequence was organized into a regular format by the FinchTv program, a comparison was made with other 16S rDNA genes found in the gene pool using the BLAST program on the NCBI website. The results were evaluated according to the findings of the comparison. Phylogenetic analysis: the 16S rRNA gene sequences of the isolate from soil was used in the phylogenetic analysis. The phylogenetic analysis was performed via the Neighbor-Joining method and carried out with MEGA 5.0 software [17]. The reliability of the phylogram was tested by the bootstrap analysis of 1000 replicates using MEGA 5.0.

Quantitative Enzyme Activity

In this study, the enzyme production capacity of the isolate, which gave a positive result as a result of the qualitative determination, was determined by the DNS method [18]. Enzyme activities were calculated with the 1 and 2 formulas;

$$\text{Enzyme Activity}\left(\frac{\text{U}}{\text{mL}}\right)=\text{Amount of reducing sugar(g)}\times\left(\frac{\text{1 mL}}{\text{Amount of sample taken}}\right)\times\left(\frac{\text{1 min}}{\text{Reaction time (min)}}\right) \quad (1)$$

$$\text{Specific Activity}\left(\frac{U}{mg}\right) = \text{Activity}\left(\frac{U}{mL}\right) / \text{Protein}\left(\frac{mg}{mL}\right) \quad (2)$$

Optimization of Production Conditions

Based on the CMC Broth enzyme production medium, the cellulase enzyme production ability of the bacteria was examined at different pH (5.0, 7.0, and 9.0), different temperatures (30, 45 and 60 °C) and different times (24, 48 and 72 hours). Enzyme-producing bacteria inoculated at the rate of 1% in media with 3 different pH values prepared with HCl and NaOH buffers were incubated at different temperatures. At the end of the incubation, the supernatants were obtained and the enzyme activities were evaluated by the DNS method [18].

Production and Partial Purification of Cellulase Enzyme

First, the cellulase producing isolate was incubated in 16-18h Nutrient Broth (NB) medium at 37°C. It was then inoculated at 1% in enzyme production medium [(CMC Broth; tryptone (10g/L), CMC (10g/L), yeast extract (5g/L), NaCl (15g/L)] and incubated at 37°C and 150 rpm for 16-18 h. As a result of the incubation, the supernatant was obtained by centrifugation at +4°C, 7000 rpm for 15 minutes. This supernatant obtained was sterilized using a 0.2 µm pore diameter filter. The sample obtained in this way was used as a crude enzyme solution (enzyme-containing supernatant) [19]. The crude enzyme solution obtained was precipitated at different (30%, 50%, and 80%) concentrations by ammonium sulfate precipitation and the activities of precipitates were determined by DNS method. The Lowry Method was used to determine the amount of protein (partially purified) of precipitates [20].

Kinetic Parameters

In this study, Vmax and Km values of the crude enzyme solution were calculated. In order to determine the enzyme kinetics, CMC substrate solutions were prepared in different concentration range (0.01-2 mM) at optimum pH value and optimum temperature value and cellulase activity was examined by DNS method. The obtained values were used to determine the Vmax and Km values of Cellulase, using the Lineweaver-Burk chart.

Optimum Activity Conditions of Enzyme

Determining the Optimum pH Value

In this study, the different substrate solutions were prepared in the pH range of 3-10 containing 1% CMC. The pH values were prepared using 0.1 M Glycine-HCl (pH 3, 4, 5 and 6) and 0.1 M Tris-NaOH (pH 7, 8, 9 and 10) buffers. The different pH values were used for enzyme activity test. The enzyme activity was measured by the DNS method [18].

Determination of pH Stability

The crude enzyme solution and the CMC substrate (pH 6.0) were incubated for different times (30, 40, 50, 60 and 120 minutes) and the enzyme activity was determined by DNS method [18].

Determining the Optimum Temperature

In this study, the temperature at which the enzyme showed the highest activity was determined by using the crude enzyme solution. For this purpose, the different temperature values (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100°C) were used for enzyme activity test. The enzyme activity was determined by DNS method [18].

Determination of Temperature Stability

For temperature stability; the crude enzyme solution was incubated for different times (30, 40, 50, 60 and 120 minutes) at 50°C, the temperature at which they showed the best activity, samples were taken at different times and the enzyme activity was determined by the DNS method [18].

SDS-PAGE and Zymogram Analysis

SDS-PAGE (12%) containing 0.1% CMC was used to determine the molecular weight of the enzyme. Electrophoresis was performed on the partially purified enzyme solution that was not exposed to the

denaturation agent. After electrophoresis, the gel was divided into two parts and a portion of the gel was stained with 0.1% Coomassie Brilliant Blue R 250 and then purified in a methanol-acid-water (5:1:4) solution. The other part of the gel was left for 30 minutes to separate the SDS from the gel in 0.1M Sodium acetate (pH 5.2) buffer containing 0.1% Triton X-100. Renaturation of the protein was achieved by incubation for 1 hour in Sodium acetate buffer without triton X-100. After this process, the zone formed around the protein band was visualized [21].

Effects of Cellulase Enzyme on Cotton Fiber

The enzyme sample (200 mL) obtained under the optimum production conditions was applied on the denim fabric with the help of a mixer at 55°C for 30 minutes in a beaker. After the application, visual control was made and imaging was with the Scanning Electron Microscope (SEM; FEI, Quanta FEG 250).

RESULTS

The isolate named DU-1 was determined to be a good cellulase enzyme producer in culture medium containing tryptone 1%, yeast extract 5%, NaCl 1% and CMC 1% (Figure 1).

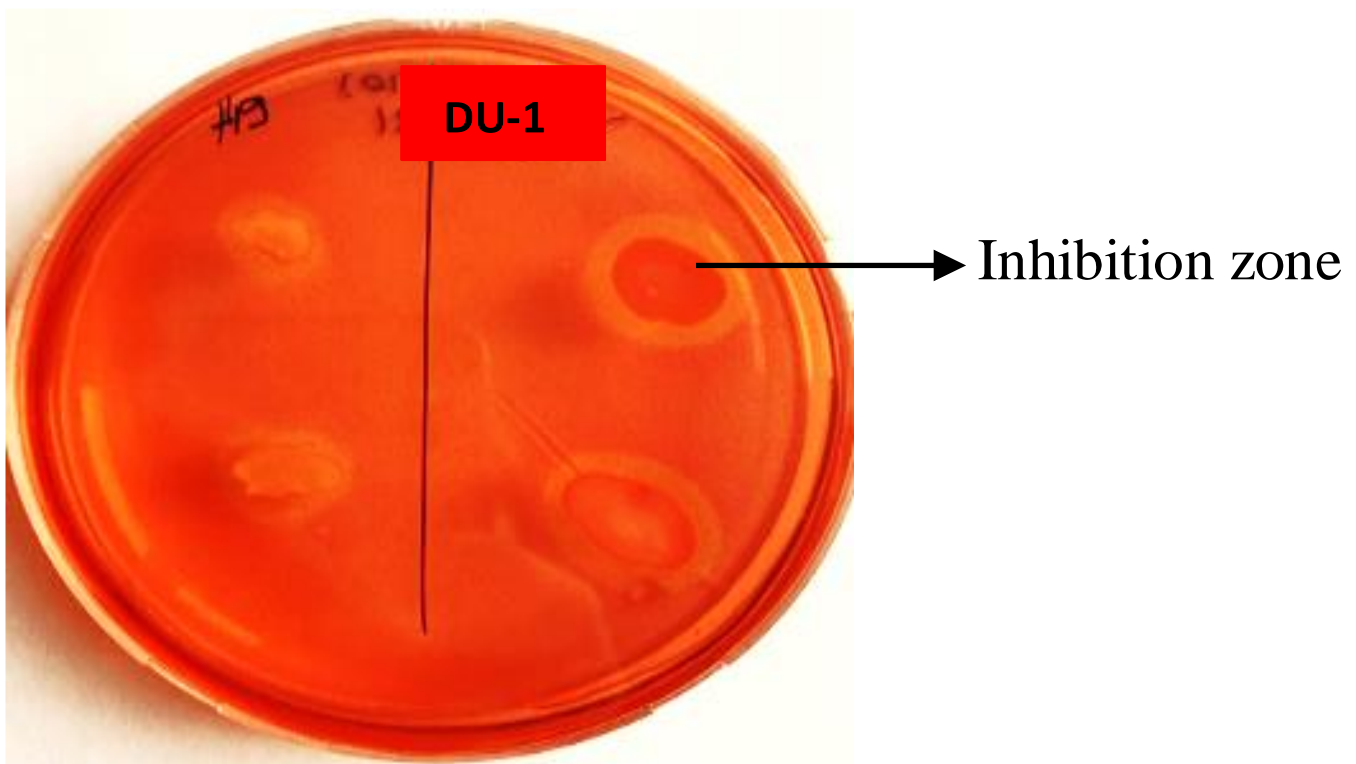


Figure 1. Qualitative determination of cellulase activity.

Identification of Bacteria

Bacterium isolated was determined by the analyzes that the bacterium was Gram (+), aerobic, rod-shaped and endospore forming. It has not crystal protein. It has a beta hemolytic activity. When all the data obtained by morphological, biochemical and molecular identification techniques were evaluated, it was determined as *Bacillus cereus*. It was named *B. cereus* DU-1 (Figure 2).

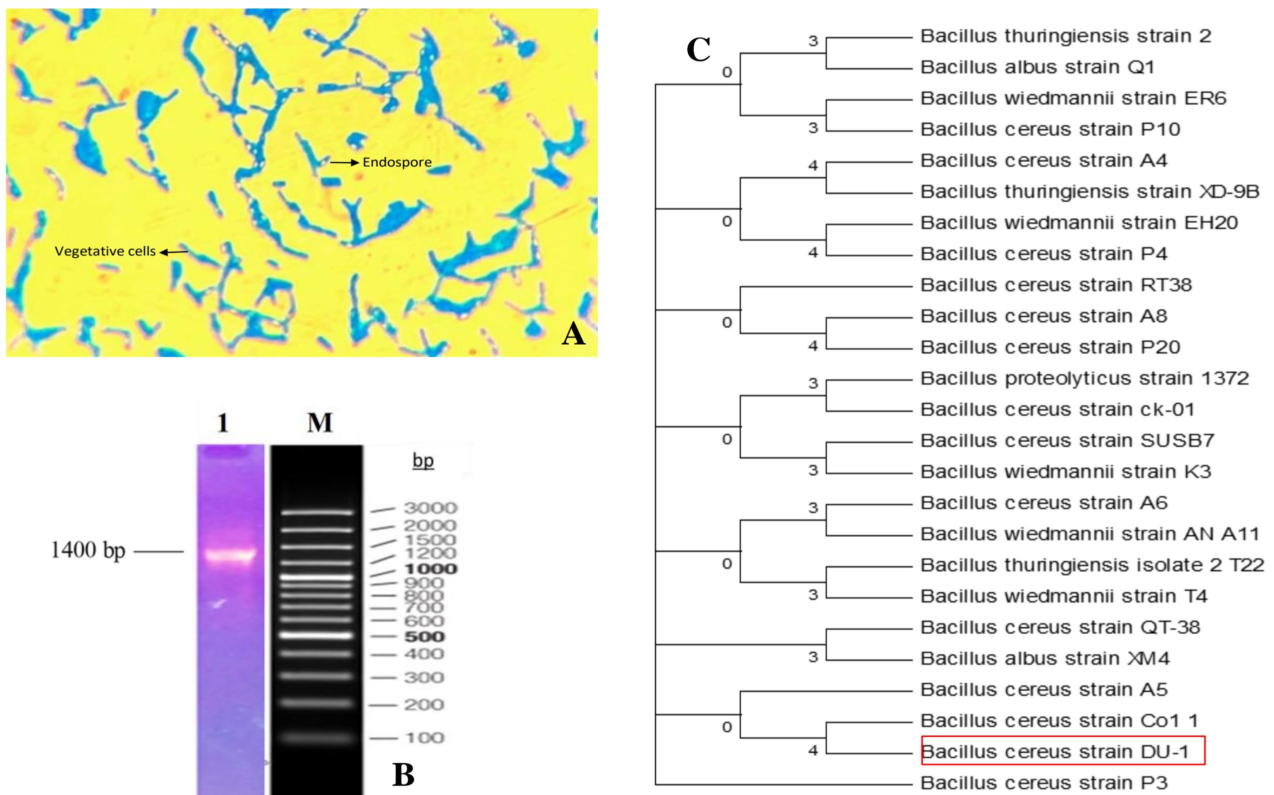


Figure 2. A - Cell morphology of the bacterium, **B -** Agarose gel electrophoresis of 16s rDNA gene of the bacterial isolate DU-1. **C -** Molecular phylogenetic analysis of bacterial isolate DU-1 by maximum parsimony method.

Optimum Production Parameters

Considering the effect of pH on the enzyme production ability of *B. cereus* DU-1 bacterium, it was determined that the best activity value was at pH 7.0 (Table 1). Considering the effect of different temperatures on the enzyme production ability of *B. cereus* DU-1 bacteria at the determined optimum pH 7.0, it was determined that the best activity value was found at 37ºC and as a result of incubation for 24 h (Table 2).

Table 1. Effect of pH on enzyme production

Temperature (ºC)	pH	Activity (U/mL)		
		24 h	48 h	72 h
30	5.0	2.80	2.60	2.00
	7.0*	3.30	2.80	3.00
	9.0	2.50	2.40	2.20
45	5.0	3.60	3.47	3.20
	7.0*	3.64	3.50	3.31
	9.0	2.79	2.96	3.00
65	5.0	1.80	1.70	1.40
	7.0*	2.00	1.80	1.50
	9.0	1.40	1.00	0.90

*; optimum pH

Table 2. Effects of temperature and time on enzyme production

Temperature (ºC)	Activity (U/mL)		
	24 h*	48 h	72 h
30	3.30	2.80	3.00
37*	3.80	3.74	3.62
45	3.60	3.50	3.31
65	2.00	1.80	1.50

***;** optimum temperature and time

Characterization of Cellulase Enzyme

Determination of Kinetic Parameters

The kinetic values of the cellulase enzyme were determined by Lineweaver-Burk plot. The Vmax value was calculated as 3.18 U/mL and the Km value was calculated as 0.0019 mM.

Optimum Temperature and Temperature Stabilization

Different temperature experiments were carried out to determine the effect of temperature on the enzyme and it was found that the enzyme had maximum activity at 50ºC. (Figure 3A). Then, the stabilization of the enzyme was examined at 50ºC, where the enzyme showed maximum activity. The enzyme solution (enzyme + substrate) was incubated in a water bath for 30, 40, 50, 60 and 120 minutes and it was observed that the enzyme remained active until the 50th minute (Figure 3B).

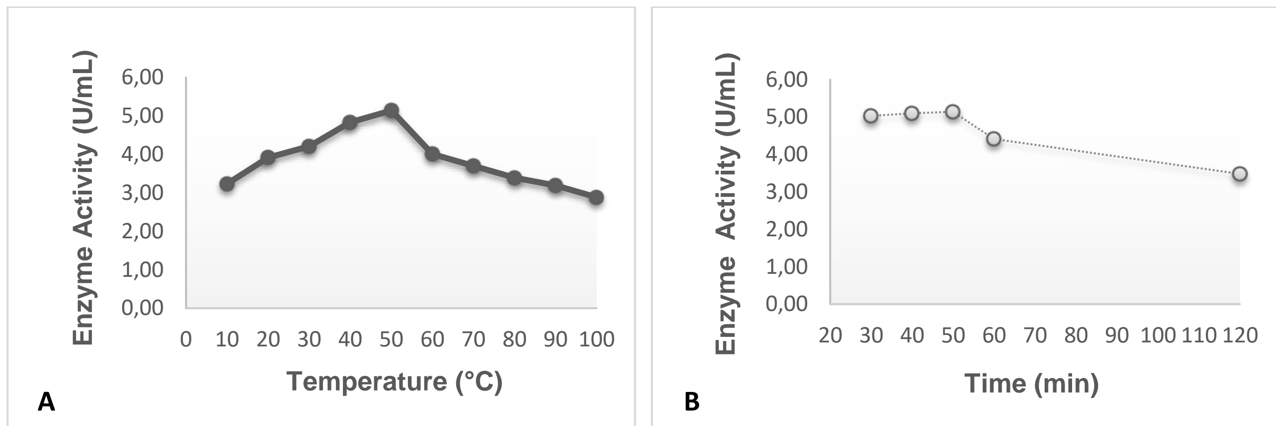


Figure 3. A - Effect of temperature on enzyme activity. **B -** Enzyme stability at 50ºC.

Optimum pH and pH Stabilization

As a result of this study carried out to determine the optimum pH value of the cellulase enzyme, it was determined that the pH value at which the enzyme showed optimum activity was 6.0, as seen in Figure 4A. To determine the pH stability of the cellulase enzyme, the enzyme was incubated in a water bath at pH 6.0 for 30, 40, 50, 60 and 120 min. While the enzyme remained active until the first 40 minutes, it was found that the activity of the enzyme was lost with the increase of the time (Figure 4B).

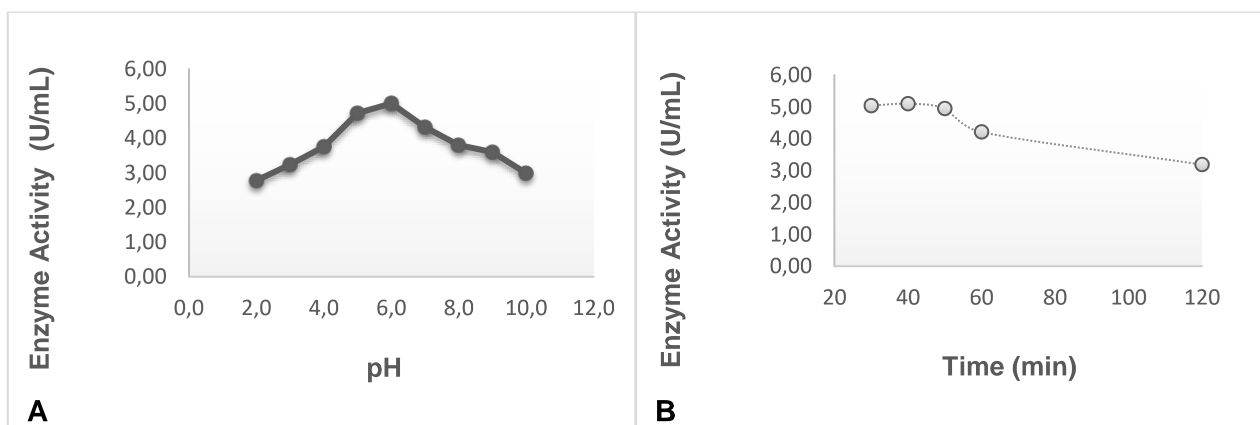


Figure 4. A - Effect of pH on enzyme activity. **B -** Effect of pH 6.0 on enzyme stability.

Production and Partial Purification of Cellulase Enzyme

In the partial purification process, precipitation was carried out in the presence of ammonium sulfate at 30% (2.53 U/mL), 50% (2.90 U/mL) and 80% (3.44 U/mL) concentrations and enzyme activity, respectively. As a result of this study, the concentration of ammonium sulfate at which the enzyme precipitated best was

determined as 80%. The activity values of the supernatant enzyme solution and the enzyme solution obtained as a result of 80% APS precipitation were calculated (Table 3).

Table 3. Partial purification of cellulase enzyme

Purification	Volume (mL)	Total protein (mg)	Total Activity (U/mL)	Specific Activity (U/mg)	Yield[1] (%)	Purity[2]
Raw enzyme	200	2.31	5.16	2.23	1.5	1
APS precipitation (80%)	10	1.76	3.44	1.95	1.95	3

[1]Yield was calculated over total activity.
[2]Purity was calculated based on specific activity.

Determination of the Molecular Weight of the Enzyme

As a result of electrophoresis, the molecular weight of the enzyme was determined as approximately 40 kDa (Figure 5A). By zymogram analysis of the enzyme whose molecular weight was determined, its activity was measured and the band was confirmed (Figure 5B).

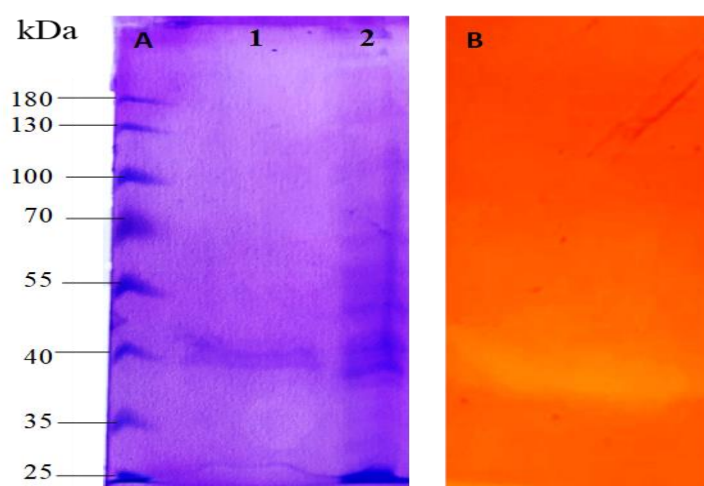


Figure 5. Determination of the molecular weight of the enzyme. **A.** PAGE image. 1; $(NH_4)_2SO_4$ precipitation (80%) 2; Crude enzyme, **B.** Zymogram analysis.

Effects of Cellulase Enzyme on Cotton Fiber

As a result of this study, which was carried out to determine the effect of cellulase enzyme on cotton fiber, it was observed that the enzyme cleans the surface and creates a smoother and softer surface in the images taken by SEM, as seen in Figure 6.

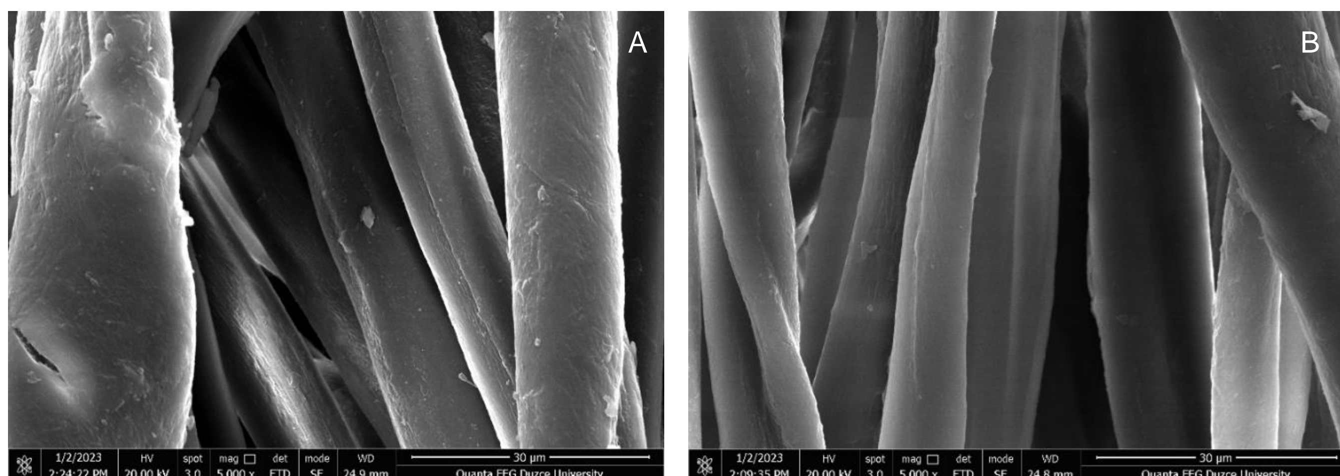


Figure 6. Determination of the effect of the enzyme on cotton fabric with SEM. **A.** Untreated cotton fabric **B.** Enzyme treated cotton fabric.

DISCUSSION

Nowadays, scientific researches with industrial enzymes are of great importance due to the gradual development of enzyme technology, the high economic value of the products and the diversity of their usage areas [1]. Enzymes needed in industrial applications are protease, amylase and cellulase, respectively. Among the enzymes used industrially, cellulase enzymes seem to be the third most important biotechnological enzyme industrially after proteases and amylases.

It has been revealed by many studies that cellulase enzymes have a wide range of applications such as food, agriculture, textile, paper, pharmaceutical and chemical industries. This study was initiated to add a new one to the existing cellulase enzymes with such a wide sectoral use, and 30 bacteria were isolated from the soil sample containing cellulotic wastes and it was determined that the isolate coded DU-1 was a cellulase producer. This isolate was determined to be *Bacillus cereus* by identification studies. *B. cereus* is also named as DU-1 strain.

Since bacteria produce the cellulase enzyme extracellularly in abundance, they are easily extracted. This makes them cellulase producers that facilitate purification [22]. *Bacillus* genus is remarkable for such hydrolytic enzymes and is frequently used in industrial processes [23]. Cellulolytic enzymes in *Bacillus* species have been well characterized. In many studies, it has been determined that bacteria belonging to different *Bacillus* species such as *B. subtilis* JBS250 [23], *B. subtilis* LFS3 [24], *B. subtilis* CBS31 [25], *B. halodurans* IND18 [26], *B. subtilis* BC1 [27], *B. subtilis* BY-4 [28], *B. subtilis* CD001 [22], *Bacillus* sp. CH43 and HR68 [29], *Bacillus* sp. ARO3 [30] and *B. vallismortis* RG07 [31] are cellulase producers.

In a study on cellulase synthesis of *Bacillus polmyxa*, it was reported that cellulase is an extracellular enzyme and the enzyme can be extracted from cells [32]. In another study, it was shown that *Bacillus brevis*, *B. firmus, B. licheniformis, B. pumilus, B. subtilis, B. polmyxa* and *B. cereus* have a cellulolytic activity [33]. *Bacillus* genus is quite remarkable among cellulase-producing bacteria, thanks to its ability to easily cultivate bacteria belonging to the genus *Bacillus* and to produce extracellular enzymes in vitro. In this context, it is foreseen in this study to obtain cellulase from a bacterium of the genus *Bacillus*. Although there are many studies on cellulase production with *Bacillus* species in the literature, there are very few studies on cellulases produced by *B. cereus* strains.

Enzymes are generally in a globular structure and are kept in a secondary or tertiary structure by intramolecular or intermolecular bonds. Protein structures can deteriorate due to changes in temperature and pH. Therefore, the catalytic activity of the enzyme is sensitive to pH and temperature [34]. It is known that the catalytic activity of the enzyme increases at optimum temperature and pH values. In this study, the effects of temperature and pH on the production and catalytic activity of cellulase enzyme produced by *B. cereus* DU-1 strain were evaluated. As a result of the study, it was determined that the enzyme was best produced by DU-1 strain in the presence of tryptone as a nitrogen source, 24 hours, pH 7.0 and 37°C. At the same time, it was determined that the cellulase enzyme produced by DU-1 strain showed optimum activity at pH 6.0 and 50°C. As a result of temperature characterization analyzes of cellulase enzyme produced by DU-1 strain, it was observed that it has thermotolerant properties. It was observed that the cellulase enzyme produced by *Bacillus cereus* DU-1 strain was thermotolerant as a result of temperature characterization analyzes and maintained its activity for 50 minutes as a result of the temperature stability test. And again, in the pH stability experiment conducted for 120 hours, it was observed that its activity was preserved for 40 minutes.

In a study, the cellulase activity of *Bacillus subtilis* BY-3 strain isolated from Tibetan boar feces was determined, and they reported that the maximum cellulase production of the bacterium occurred after 24 hours when corn cob was used as a substrate. In addition, the researchers reported that the optimum enzyme activity was realized at pH 5.5 and 60 ºC temperature values, and that it had the best stability as a result of preincubation at 60 ºC for 60 minutes [35]. In a different study, it was reported that the thermostable alkaline cellulase isolated from *Bacillus* sp. KSM-S237 showed its optimum activity at pH 8.6-9.0 and 45°C [36]. In another study by Kim and coauthors, alkaline cellulase isolation was performed by *Bacillus* sp. HSH-810 strain and it was reported that the enzyme showed optimum activity at pH 10.0 and 50°C [37]. They found that the optimum operating temperature of the endoglucanase enzyme they obtained from *B. licheniformis* was 65 ºC, they explained that it was more stable at 60ºC in a 1 hour incubation process and had more than 90% activity. The enzyme, which has an optimum working pH of 6.0, has 40% activity at pH 10.0 [38]. In another study, it was reported that the highest level of cellulase production by thermophilic *Bacillus* was reached at 48 hours and 45 ºC, and the optimum pH range for enzyme activation was 6.5-7.5 [39]. In a different study, it was reported that optimum temperature and pH conditions for the production of cellulase enzyme produced by *Bacillus* sp. C1AC5507 were 70 ºC and 7.0, respectively [40]. In another study, it was found that the cellulase enzyme produced by *Bacillus subtilis* SU40 showed maximum activity at pH 8.0, the

enzyme preserved more than 50% of its activity in the pH range of 7-9 for 30 minutes, the enzyme maintained its activity in the temperature range of 35°C-75°C, and it has been reported that the temperature at which its activity is highest is 45°C [41]. The optimum temperature for cellulase enzyme activity produced by *Bacillus vallismortis* RG-07 was determined as 65°C and pH 7.0. It has been observed that it maintains 95% and 75% of its activity even at 95°C and pH 9.0 [31]. It was determined that cellulase enzymes produced from two *Bacillus* strains showed their optimum activities at pH 5.0-6.5 and at 65 and 70°C [29]. It was found that alkaline endoglucanase isolated from *Bacillus circulans* works optimally at pH 8.5 at 55°C [42]. As can be understood from the research, there are enzymes that are similar to the optimum conditions of the cellulase enzyme obtained in this study [39]. As well as enzymes that are active and produced under quite different conditions [40].

Cellulase enzyme kinetics of *B. cereus* DU-1 strain were investigated and Vmax and Km values were found as 3.18 U/mL and 0.0019 mM, respectively. It is known that soil cellulases are variable and therefore cellulases from different soils may show different Km and Vmax values [43]. In a study, Km and Vmax values of the cellulase enzyme produced by *Bacillus subtilis* SU40 were reported as 1.97 mg/mL and 75.41 mg/mL/s [41]. They reported that the Km and Vmax of the cellulase enzyme produced by *Bacillus vallismortis* RG-07 bacteria were 1.923 mg/mL and 769.230 µg/mL, respectively [31]. When compared with other studies, it is seen that the Km value of the cellulase enzyme characterized in this study is quite low and the substrate affinity is extremely high. In addition, the fact that the Vmax value is quite high gives the information that the catalytic power of the enzyme is high.

In this study, the supernatant containing active enzyme was precipitated by 80% $(NH_4)_2SO_4$ and partially purified. As a result of SDS-PAGE and zymogram analysis studies, it was determined that the cellulase enzyme produced by *B. cereus* DU-1 strain was 40 kDa in size. In a study, cellulase enzyme produced by *Bacillus vallismortis* RG-07 was purified by $(NH_4)_2SO_4$ precipitation, ion exchange and gel filtration chromatography methods. Afterwards, the molecular weight of the purified cellulase was determined as 80 kDa by SDS-PAGE and activity gel analysis [31]. The molecular weight of cellulase purified from *Bacillus licheniformis* bacteria has been reported as approximately 55 kDa [44]. The molecular weight of the cellulase enzyme produced by *Bacillus* sp. C1AC5507, which is also a thermophilic strain, has been reported as 55 kDa [40]. The cellulase enzyme produced by *Bacillus subtilis* SU40 was reported to have a molecular weight of 51.4 kDa by SDS-PAGE [41]. In a different study, as in our study, the molecular weight of cellulase enzymes produced from two *Bacillus* strains was reported as 40 kDa [29]. In another study, it was determined that alkaline endoglucanase isolated from *Bacillus circulans* had a molecular weight of 43 kDa [42]. The molecular weight of alkali cellulase enzyme obtained from *Bacillus sphaericus* JS1 strain was determined as 42 kDa [45]. In this study, it is seen that the cellulase enzyme obtained from *B. cereus* DU-1 has a similar molecular weight with the cellulases synthesized by some *Bacillus* species [29,45].

CONCLUSION

As a result of this study, *Bacillus cereus* was isolated, and it was determined that it is also a good producer of cellulase enzyme. The fact that the enzyme is temperature tolerant indicates that it may be important for industrial use. In future studies, it is very important to design the enzyme production environment as cellulolytic agricultural wastes and to reveal the potential of using the enzyme, which is determined to have high substrate affinity, at low cost and to be used in various industrial areas. In this context, the cellulase enzyme produced was treated on the denim fabric. After the application on the denim fabric, electron microscope images were taken and it was observed that the enzyme has the potential to wear the fabric in the images zoomed 500 times. In this way, with this study, it has been shown that this environmentally friendly enzyme, which the textile setter has already used for surface bleaching, biopolishing and hair removal, can be successfully obtained from *B. cereus*.

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