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Purification And Analysis of The Properties of The Prostheses Enzymes From Bacillus subtilis Megatherium Bacteria

Bacillus Subtilis Megatherium Bakteri Türünden Proteaz Enziminin Saflaştırılması ve Özelliklerinin İncelenmesi

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ABSTRACT

Enzymes are biocatalysts produced by living organisms that affect the rate of critical biological reactions. Enzyme technology is developing rapidly today and is gaining importance due to the products' diversity and high economic value. The most important enzyme used in this field is a protease. Proteases are widely used in detergent, food, pharmaceutical, and leather industries. More than 60% of enzymes are of microorganism origin. Our study aimed to purify the protease enzyme from *Bacillus subtilis megatherium* and to determine its properties. In the first step of the study, the enzyme was produced under suitable conditions. Dialysis, ammonium sulfate precipitation, and DEAE-Sefarose ion exchange chromatography methods were used for Purification. The specific activity of the crude homogenate was found to be 10.2 U/mg. After 80% precipitation, the solution-specific activity was 12.9 U/mg. After saturation, 1.2-fold Purification was achieved. The optimum temperature, temperature stability, optimum pH, and pH stability of the protease enzyme, the effect of inhibitor and activator on the reaction, the duration of the reaction, and the effect of the substrate were examined, and the molecular weight was determined.

Anahtar Kelimeler: Alkaline protease, *Bacillus subtilis*, Purification

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ÖZET

Enzimler canlı organizmalar tarafından üretilen, önemli biyolojik reaksiyonların çalışma hızını etkileyen biyokatalizörlerdir. Enzim teknolojisi günümüzde hızla gelişmekte olup, çıkan ürünlerin çeşitliliği ve ekonomik değerlerinin yüksek olması sebebiyle hızla önem kazanmaktadır. Bu alanda kullanılan en önemli enzim proteazdır. Proteazlar deterjan, gıda, ilaç ve deri sanayisinde çok sık kullanılmaktadır. Enzimlerin %60'ından büyük bir payını mikroorganizma kaynaklıdır. Çalışmamızın amacı Bacillus subtilis megatherium bakterisinden proteaz enziminin saflaştırılması ve özelliklerinin belirlenmesidir. Çalışmanın ilk basamağında enzimin uygun şartlarda üretimi sağlanmıştır. Saflaştırma için diyaliz, amonyum sülfatla çöktürme ve DEAE-Sefaroz iyon değişim kromatografi yöntemleri yapılmıştır. İşlemler sonrasında ham homojenat spesifik aktivite 10,2 U/mg bulunmuştur. %80'lik çöktürme işleminden sonra çözelti spesifik aktivitesi 12,9 U/mg' dır. Doyurma sonrası 1,2 kat saflaştırma sağlanmıştır. Proteaz enziminin optimum sıcaklık, sıcaklık kararlılığı, optimum pH ve pH kararlılığı; reaksiyon üzerine inhibitör ve aktivatör etkisi, reaksiyonun süresine ve substratın etkisine bakılmış olup molekül ağırlığı tayini yapılmıştır.

Keywords: Alkaline proteaz, *Bacillus subtilis*, Saflaştırma

1. Introduction

Enzymes are specialized biocatalysts, mostly in protein structure, that regulate these reactions and ensure that occurring in living organisms occur under appropriate conditions. Enzymes, which have essential metabolic functions in the structure of cells, have been used by humans for many years. For example, enzymes produced by organisms are used in the production of bread, vinegar, boza, wine, and yogurt. Enzymes are used in food production, cleaning materials, and medical diagnosis and treatment (1).

Enzymes are replacing chemical processes in many industrial fields because they cause less environmental pollution and can be realized economically. Alkaline protease enzymes have a 60% share among industrial enzymes (2). Enzymes must first be purified for their use in industry and technology. Microorganisms are used as enzyme sources due to their easy reproduction and easier Purification of the enzymes they produce. The fact that most enzymes work at low temperatures and in a narrow pH range causes them not to be used in the industry's wide pH range and high-temperature conditions. For this reason, it encourages the discovery of new enzymes that can meet the needs of the industry and microorganisms that can produce these enzymes. Microorganisms are an essential source for obtaining enzymes (3).

Many extremophile microorganisms, including the *Bacillus genus* and *Bacillus genus* species, are widely used in protease production (4). It is used in the health, food and leather industries, especially detergent industries. It is also used in many industrial fields, such as the hydrolysis of proteins such as feathers and hair and converting these proteins into valuable biomass (5,6).

2. Material and Method

Our study used Dialysis-Centrifugation-Precipitation-Ion Exchange Chromatography as separation and enzyme purification methods. The SDS-PAGE method was used to determine the molecular weight of the purified enzyme.

Medium Preparation and Inoculation: Liquid medium was prepared by taking 3 g of meat extract and 5 g of peptone and adding distilled water to 1 liter. pH was determined as 7 with 1M Na_2CO_3. To increase the sporulation rate, 10 mg MnSO4.2H2O was added to the liquid medium. Solid medium and curved solid medium were prepared in petri dishes by taking 5 g of bacto soybean, 5 g of sodium chloride, 15 g of bacto tryptone, 15 g of bacto agar, adding distilled water to 1 liter and adjusting the pH to 7.3. The media were sterilized in an autoclave at 121°C for 15 minutes. Dried *Bacillus* species were obtained in ampoules. The breakage process was applied to the ampoules. As a cracking process, the end of the ampoule was scratched, heated, and cracked by the sudden addition of water. After this process, a small amount of liquid collected from the liquid medium with a pipette was injected into the ampoule and suspended. All of the suspended liquid was removed and added to the liquid medium. Next, the solid medium was inoculated into the petri dish using the reduction method. The back side of the Petri dish was divided into four with a glass pen and numbered clockwise. Bacteria taken from the liquid medium with the help of a needle were inoculated by drawing zigzags in the areas divided into four, respectively. After inoculation, the petri dishes were kept in an oven at 37°C for 24 hours for growth. The pure culture was obtained from the growing colonies. For culture, bacteria taken from a single colony with a needle were inoculated on curved solid medium and kept in an oven at 37°C for 24 hours. Samples were taken and inoculated on a different curved solid medium. After 48 hours, the sample was inoculated into the previously prepared liquid medium. Sporulation and growth were monitored under appropriate growth conditions.

Precipitation with Ammonium Sulfate: The solution and bacteria are separated by centrifuging the medium at 20000 rpm for 30 minutes. The precipitated part is separated. Proteins in the solution are separated by ammonium sulfate precipitation of the liquid part, which is crude homogenate. Precipitation takes place in three parts. In the first part, 40% of precipitation took place. The amount of ammonium sulfate added for the level (ml) of crude homogenate in 100 ml solution containing 25 g ammonium sulfate was calculated for the precipitation process. Precipitation was carried out at 4°C. After adding ammonium sulfate, the solution was stirred for half an hour and then centrifuged at 20000 rpm for half

an hour. After centrifugation, the precipitate and solution (supernatant) were separated. The solution was identified as A1 and the precipitate as B1. For protein and activity determinations, 5 ml of A1 was separated and placed in the refrigerator with B1. Precipitation was carried out with A1. The second part was 70% ammonium sulfate saturation. In this part, 100 ml of a solution containing 44.2 g of ammonium sulfate was made. The measure of ammonium sulfate calculated for solution A1 and the measure of ammonium sulfate used for the 40% precipitation was subtracted from this value to find the measure of ammonium sulfate added in the 70% precipitation. The precipitation sections were all performed at 4°C. The solution to which ammonium sulfate was added was stirred for half an hour. The solution was then centrifuged at 20000 rpm for half an hour. After this process, the precipitate and solution (supernatant) portions were separated. The solution portion was labeled A2, and the precipitate portion was labeled B2. For protein and activity determinations, 5 ml of A2 was separated and refrigerated with B2. The processing of the solution was continued with A2.

In the 3rd part of the precipitation, 80% ammonium sulfate precipitation was applied. A solution of 52.3 g ammonium sulfate 100 ml was prepared. The amount of ammonium sulfate was calculated, and an A2 solution was made. Subtracting from this the total amount of ammonium sulfate used in the 70% and 40% precipitation, the amount of ammonium sulfate required for the 80% precipitation was found. All parts of the precipitation were carried out at 4°C. The solution to which the ammonium sulfate was added was stirred for half an hour. The solution was then centrifuged at 20000 rpm for half an hour. After this process, the precipitate and solution (supernatant) portions were separated. The solution portion is A3, and the precipitate portion is B3. A3 and B3 were refrigerated for protein and activity assays. After the activity determination, the expected values were found to be in the precipitates, and the procedures were continued in the precipitates.

Dialysis At the end of the precipitation phase, all the precipitates were collected, and studies were continued on the total precipitates. Dialysis was performed on the total precipitate. Dialysis tubes were conditioned before the procedure. Glycerin was removed from the tubes by washing them with water for 3-5 hours. In addition, sulfur components were removed by boiling in sodium sulfite (0.3% Ag./vol.) solution at 80°C for 60 seconds. It is contacted with a sulfuric acid solution (0.2% v/vol.) for a while by washing with hot water at 60°C for 2 minutes. In the last step, the acid is removed by washing it with hot water. After conditioning, specific amounts of the samples to be dialyzed were placed in tubes and dialyzed with the appropriate buffer at 100 times the sample amount. Dialysis time was supplemented with Nessler Reagent. After dialysis, the samples were centrifuged. Protein and activity determinations were made before and after centrifugation.

Ion Exchange Chromatography: First, the enzyme solution is passed through a DEAE- Sefarose ion exchanger. The column used has a diameter of 2 cm and a height of 30 cm. The column was washed with the appropriate buffer at 3ml/min for half an hour. The column was fed with 10 ml of the sample, and the flow rate was adjusted to 1ml/min. After feeding, the sample was passed through the column with NaCl buffer for 2.5 hours. All samples were stored in the refrigerator for activity and protein determination.

Activity Determination: All our studies were performed using two activity determination methods. As a result of the methods, a trosin standard curve graph was created. For this, 21 mg trosin with a concentration ratio of 0.2 M was dissolved in 100ml TCA solution, and standard solutions were made by diluting the required ratio.

Activity Determination Method 1: All activity measurements were performed at 35°C. The water bath was also adjusted to 35°C. First, 3 ml of Milk Casein Substrate Solution 1 was kept in the water bath for 5 minutes. Then 0.5 ml of the enzyme solution was added, and the reaction was allowed to occur for 10 minutes. After the time was completed, 3.2 ml of TCA Mixture Solution 1 was added and kept for 10 minutes to finish the reaction. All steps were performed except the addition of enzyme solution in the witness sample. The samples were filtered through blue band filter paper, and absorbance values were determined in a spectrophotometer at 275nm.

Activity Determination Method 2: All activity measurements were performed at 30°C. The water bath was also set to 30°C. First, 2.5 ml of Milk Casein Substrate Solution 2 was kept in the water bath for 5 minutes. Then 0.5 ml of the enzyme solution was added, and the reaction was allowed to occur for 10 minutes. After the time was completed, 2.5 ml of TCA Mixture Solution 2 was added to finish the reaction and kept for about half an hour. All steps were performed except the addition of enzyme solution in the witness sample. The samples were filtered through blue band filter paper, and absorbance values were determined in a spectrophotometer at 275nm.

Protein Determination: UV Absorption and BCA Method were applied for protein determination

calculations. Since the results of the studies were close, we continued with a single study and continued the studies with the UV Absorption Method. This method calculated protein amounts (mg/ml) of enzyme samples at 280 nm. A working buffer was used as a witness sample. Protein amounts were shown using BSA standard curve.

3. Results

Study 1: *Bacillus subtilis megatherium* liquid medium was inoculated, and the flasks were placed in an oven at 37°C for sporulation and growth. At the end of 2 days, 5 ml samples were taken in the morning and evening at 12 hours intervals. After 156 hours, sampling was discontinued. All samples were centrifuged, and the solution portions were placed in the refrigerator for Protein and activity determination. The solutions in the urn were centrifuged, and the precipitate and solution were separated. They were kept in the refrigerator for 48 hours for protein and activity measurements. Activity Determination Method 1 was used in the study. For high protein values, samples were diluted 1/20 with Sodium-Borate Buffer 2 (0.01mol/l; pH: 10.5) and measured. The measurement results are shown in Table 1 and Figure 1. In Study 1, individual measurements were made on the samples from the four separated flasks. According to the measured results, the highest activity was at 96th and 156th hours. 96 and 156 hours are the most suitable times for sporulation and reproduction.

Table 1. *Bacillus subtilis megatherium* specific activity-time values (Q1)

Time (hours)	Activity (U/ml)	Protein (mg/ml) (1/20)	Specific Activity (U/mg)
48	159,2	0,718	8,9
60	145,9	0,59	10,5
72	132,3	0,535	10,6
84	120,3	0,528	10,5
96	112,7	0,468	12,3
108	107,8	0,485	10
120	108,9	0,463	10,5
132	106,7	0,475	10,3
144	111,4	0,477	10,9
156	119,1	0,489	12,6
168	115,6	0,521	10,6

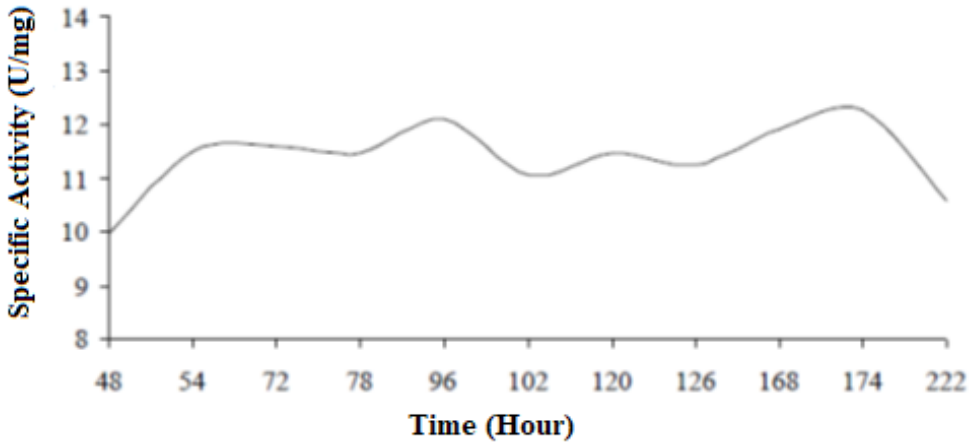

Figure 1. *Bacillus subtilis megatherium* specific activity-time curve (Q1)

Study 2: In Study 2, changes in the specific activity of the protease enzyme produced from the *Bacillus subtilis megatherium* strain were examined. Samples for the investigations were started early in the day and taken at frequent intervals. The enzyme solution was subjected to precipitation and dialysis with ammonium sulfate. The medium preparation and inoculation procedure was described in section 3.5.2. Only 200 ml of the liquid medium was prepared, and 42 500 ml flasks of 100 ml each were autoclaved for sterilization.

Bacillus subtilis megatherium liquid medium was inoculated, the flasks were shaken on a room shaker at 37°C, and sporulation and growth times were observed. At the end of 2 days, 5 ml samples were taken in the morning and evening at 12 hours intervals. After 156 hours, sampling was discontinued. The samples were centrifuged, and the solution portions were placed in the refrigerator for Protein and activity determination. The solutions in the urn were centrifuged, and the precipitate and solution were separated.

They were kept in the refrigerator for 48 hours for protein and activity measurements. Activity Determination Method 1 was used in the study. For high protein values, the samples were diluted 1/20 with Sodium-Borate Buffer 2 (0.01mol/l; pH: 10.5) and measured. The data obtained are shown in Table 2 and Figure 2. By the data obtained, the samples in the flasks were centrifuged, and protein and activity measurements were performed together with the other samples collected (crude homogenate). The volume of the crude homogenate was measured and precipitated with ammonium sulfate.

Precipitation was carried out at 40%, 70%, and 80%. For precipitation, ammonium sulfate was added, and the precipitate (P1, P2, P3) formed by centrifugation was dissolved with the smallest volume of Sodium-Borate Buffer 1 (0.01mol/l; pH:9.3). After precipitation, the dialysis tubes were conditioned and dialyzed to total precipitate (P4) and 80% precipitation solution (C). 5 ml of P4 was dialyzed against 500 ml of Sodium-Borate Buffer 3 (0.01mol/l; pH: 9.3) overnight. Nessler reagent was controlled by dialysis. The volume of the dialysis sample designated Ç4X, was measured and centrifuged at 20000 rpm for approximately half an hour to separate the precipitate (P5A) and solution (Ç5X).

The Protein and activity of the samples were determined before and after centrifugation. 30 ml of Ç3 was dialyzed against 3 liters of Sodium-Borate Buffer 3 (0.01mol/l; pH: 9.3) overnight. Dialysis control was performed with the Nessler reagent. The volume of the dialysis sample, designated as P4Y, was measured and centrifuged at 20000 rpm for about half an hour to form a precipitate (P5B) and solution (P5Y). Portions of P5A and P5B were excluded. The Protein and activity of the samples were determined before and after centrifugation (Table 2) (Figure 2).

Table 2. *Bacillus subtilis megatherium*-specific activity-time values

Time (hours)	Activity (U/ml)	Protein (mg/ml) (1/20)	Specific Activity (U/mg)
48	103	0,412	12,5
60	92,5	0,378	12,1
72	92	0,376	12,1
84	90,1	0,388	11,2
96	91,6	0,386	11,1
108	99,8	0,390	12,7
120	96,8	0,401	11,2
132	102,1	0,417	12
144	97,6	0,385	12,2
156	99,8	0,389	12,1
168	97,4	0,398	11,2

Table 2 shows the specific activity-time average values of *Bacillus subtilis* megatherium. Specific activity values are around 12 U/mg at the end of 156 hours. It was observed that it started to decrease after this hour. As a resultof Study 2, a higher specific activity was obtained compared to Study 1, and a higher specific activity was obtained according to the duration.

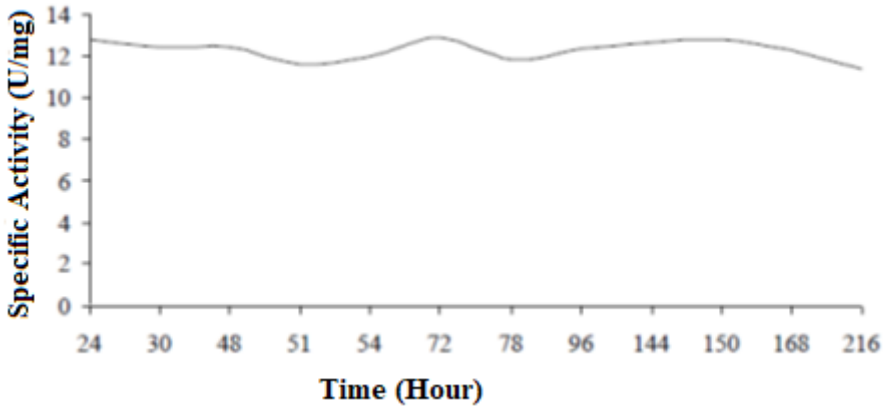

Figure 2. *Bacillus subtilis megatherium* specific activity-time curve

Table 3. Partial Purification of protease enzyme from *Bacillus subtilis megatherium*

BSM **Substrate** **Casein**	Volume (ml)	Protein (1/20) (mg/ml)	Activity (U/ml)	Total Protein (mg)	Total Activity (U)	Specific Activity (U/mg)
Raw Homogenate	124	0,397	96,5	1189	12398	9,8
40% precipitation (C1)	126,7	0,354	100	969	13199	12,9
40% precipitation (P1)	5,9	0,598	75,2	81	498	5,1
70% precipitation (Ç2)	129	0,301	81,9	942	11287	11,8
70% precipitation (P2)	5,8	0,300	50	48	348	6,9
80% precipitation (Ç3)	127	0,299	79,8	800	10547	12,8
80% precipitation (P3)	5,8	0,297	42	41,5	279	5,9
Total sediment (P4)	17,5	0,398	39	160	784	4
Dialysis post and centr, pioneer (Q4X)	6	0,201	2	29,8	15,8	0,35
After dialysis and before dialysis (Q4M)	70	0,032	1,1	50	91,8	1,2
After dialysis, and after centr, (Q5X)	4	0,089	1,3	8,1	8	0,6
After dialysis, and after centr, (Q5Y)	65	0,014	3	19	228	9,7

The specific activity of the crude homogenate was 9.8 U/mg and the specific activity of the solution at 80% precipitation was 12.8 U/mg. The specific activity of the precipitate at 80% precipitation was calculated as 5.9 U/mg. These results revealed that the enzyme mostly remained in the solution and did not pass to the precipitate in the precipitation. A 1.5-fold purification occurred in the precipitation compared to the solution. Dialysis of the precipitate and solution resulted in the loss of specific activity and no purification. When these measurements were compared using two different methods (Activity Determination Method 1: Specific Activity 12.9 U/mg and Activity Determination Method 2:12.2 U/mg), the specific activities were approximately the same. The study result is suitable for use in both methods.

Effect of pH on Enzyme Activity: In this study, optimum pH and pH stability were investigated. pH range was changed between 4-10, and measurements were made using Activity Determination Method 2. As a result of the measurements, the optimum pH value of the enzyme sample was determined as 8.97. The Tris-HCl buffer used in the study for pH stability was changed between the pH range 4-10, and measurements were made using Activity Determination Method 2. As a result of the measurements, the protease enzyme showed degradation after pH 10.

Temperature Effect on Enzyme Activity: The enzyme was subjected to different temperatures, and the optimum temperature was determined using activity determination. Activity Determination Method 2 was used for this. The water bath used was changed between 20ºC and 80ºC instead of 30ºC. At these temperatures, the effect of the enzyme samples on amino acids and how the effect on activity values changes if the enzyme loses its effect was examined. The results showed that the enzyme lost its effect at 70°C. The optimum temperature is around 60°C for the *Bacillus subtilis* megatherium strain.

In the temperature stability studies, all of the enzyme samples were placed in 0.5 ml tubes each and were oven dried at temperatures between 10°C and 80°C for 15 minutes. Then they were cooled at room temperature for 5 minutes, and activity was determined by Activity Determination Method 2. The results showed that the enzyme lost its activity at 70°C. According to the results, *Bacillus subtilis* megatherium was stable in the temperature range of 30-60°C.

Effect of Metal Ions on Enzyme Activity: In this study, the effects of metal ions on enzyme activity were investigated. One mM solution of metal ions in the investigation was prepared and measured using Activity Determination Method 2. The effects of $MnCl_2$, $FeCl_2$, and $CaCl_2$ salts on the activity of samples obtained from *Bacillus subtilis* megatherium were investigated. $MnCl_2$ salt was found to be a suitable activator. $FeCl_2$ and $CaCl_2$ salts were not good activators and had an inhibitory effect. $CuCl_2$ and EDTA salts were used as inhibitors. According to the results, EDTA was determined as a suitable inhibitor.

Effect of Reaction Time and Substrate Concentration: For this study, the time was adjusted between 15-85 min and the substrate concentration between 1-0.07%. Activity determinations were performed by Method 2. The activity values changed at different reaction times, and substrate concentrations and all concentrations were very close at 70 minutes. As a result of the measurements, substrate concentrations did not change the activity after 70 minutes.

4. Discussion and Conclusion

The optimum temperature of the enzyme sample made from *Bacillus subtilis* megatherium is 37ºC pH value 8,97. Serine proteases show high activity at this value, which is within alkaline limits. It can be said that the protease enzyme found in our study is in the serine protease group. In the studies conducted by Bhaskar et al. (2007) and Denizci et al. (2004), alkaline protease production was similar (7,8). Kaur et al. (2001) reported protease enzyme activity at 30 and pH value as 9.0 (9). Enzymes show their effects at different pHs. Enzyme activity shows significant changes at different pHs (10). In their study, Mao et al. (1992) stated that enzyme production would be controlled by determining temperature and pH values (11).

As a result of the calculation for sporulation and reproduction conditions in Study 1, high activity was calculated at 96th and 156th hours. At 168 hours and after, the activity decreased. Between 96 and 156 hours are the most suitable periods for sporulation and reproduction. In Study 2, specific activity values were around 12 U/mg at the end of 156 hours and decreased after 156 hours. Since it was done by shaking, a high and stable result was obtained compared to Study 1. As a result of the study, 156 hours were found to be sufficient time. In the Purification in Study 2, the crude homogenate-specific activity was 9.8 U/mg, and the solution-specific activity in 80% precipitation was 12.8 U/mg. In the precipitate, the specific activity was determined as 5.9 U/mg at 80% precipitation. Most of the enzyme remained in the solution and could not be transferred to the precipitate by precipitation. There was 1.5-fold Purification after precipitation compared to the solution. After the precipitate and solution were dialyzed, the specific activity was lost, and there was no purification. In related studies, alkaline proteases purified from different species showed 58%, 65%, and 24% yield and 3.9, 4.9, 21.4- fold Purification by ion exchange chromatography column output, respectively (12,13). In another study, different chromatographic procedures were performed to increase the degree of purified alkaline proteases from *B. pumilus* and *Bacillus sp.* PS719 strains. It was reported that alkaline protease was purified 9.9-fold with a 79% yield from *Bacillus clausii* I-52 strain (14).

Although the optimum temperature values of alkaline proteases isolated from *Bacillus* strains are generally in the range of 50-70ºC (15,16), they vary between 40-55ºC in some mesophilic *Bacillus* species and 70-75ºC in thermophilic *Bacillus* species (14,17). Our study's optimum temperature was around 60°C for the Bacillus subtilis megatherium strain. According to the results, Bacillus subtilis megatherium was found to be stable in the temperature range of 30-60°C. Kumar (2002) reported the optimal temperature of *Bacillus pumilus* alkaline protease as 55-60 ºC, and Kazan et al. (2005) reported the optimal temperature of *B. clausii* GMBAE 42 alkaline protease as 60 ºC (12,15). In literature studies, alkaline proteases purified from *Bacillus* species were shown to have a temperature range of 30-60ºC (15-19).

When the effect of metal ions was analyzed, it was determined that MnCl2 salt was a suitable activator. FeCl2 and CaCl2 salts were not found to be suitable activators and had an inhibitory effect. CuCl2 and EDTA salts were used as inhibitors. According to the results, EDTA was determined as a suitable inhibitor. Activity values changed at different reaction times, and substrate concentrations and all

concentrations were very close at 70 minutes. As a result of the measurements, substrate concentrations did not change the activity after 70 minutes. In previous studies, it has been reported that metal ions such as Ca2+, Mg2+, and Mn2+ have activating effect, metal ions such as Fe3+, Hg2+, Cu2+, Cu3+, Cu3+, Ag+, and Zn2+ have an inhibitory effect, and other metal ions such as Na+, K+, Co2+, Cd2+, and Fe2+ suppress the proteases very little (20-24). Beg and Gupta (2003) showed that the alkaline protease of *Bacillus marmariensis* has high activity with Cu2+ ions at 30ºC (25). Although the activator effect of Ca2+ ions in the activities of microorganism-derived proteases is known, Ca2+ and Mg2+ ions inhibit the alkaline protease of *Bacillus marmariensis*. While Fe2+ and Zn2+ metal ions have little or no inhibition effect on alkaline proteases, they cause potent inhibition on GMBE 72 alkaline protease (15, 20-24). In the molecular weight determination in our study, the molecular weight of the protease enzyme obtained from the *Bacillus subtilis megatherium* strain is around 45 kDa. Molecular weights of alkaline proteases purified from different *Bacillus* species are in the range of 15-36 kDa (26,27). Yossan et al. (2006) reported the molecular weight of alkaline protease isolated from *Bacillus megaterium* as 27 kDa, Seifzadeh et al. (2008) reported the molecular weight of protease isolated from *Bacillus sp.* as 47 kDa, Falahatpishe et al. (2007) reported the molecular weight of *Bacillus sp.* protease as 24.7 kDa (21,28,29). In line with the results, proteases cannot be generalized with their molecular weights. Proteases with different molecular weights were found in the same species, and proteases with the same molecular weight in the same species.

Although there was not enough Purification from the enzyme, it was determined that the heat and pH stability, which is essential for the industry, was high. Bacteria can be grown in various environments to determine their yield and purity. This will enable the enzyme to be used industrially, and the protease supplied from foreign markets can be produced in our country and contribute to the national economy.

Declaration of Ethical Code

In this study, we undertake that all the rules required to be followed within the scope of the "Higher Education Institutions Scientific Research and Publication Ethics Directive" are complied with, and that none of the actions stated under the heading "Actions Against Scientific Research and Publication Ethics" are not carried out.

Ethics committee approval for this study was obtained by Burdur Mehmet Akif Ersoy University Non-Interventional Clinical Research Ethics Committee on 03.04.2019 with the number 2019/72.

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