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Partial Characterization of Hydrolytic Enzymes Produced by *Bacillus* Strains Isolated from Balıklıgöl, Turkey

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ABSTRACT

In the present study, forty-five bacterial isolates were obtained from previously unstudied soil samples in Balıklıgöl, Şanlıurfa. Based on their enzyme production capacities, six bacterial isolates designated as BGL-22, BGL-26, BGL-27, BGL-37, BGL-38 and BGL-39 were selected for further studies. Conventional and molecular identification results showed that the bacteria belonged to *Bacillus* genus. Among these strains, the highest activities for amylase (11.44 U mL^{-1}), lipase (1.12 U mL^{-1}) and protease (2.61 U mL^{-1}) were determined for *Bacillus* sp. BGL-37. Enzymatic characterization studies demonstrated that the activities of acid-stable amylase and alkaline-stable lipase remained unchanged up to 50°C, while alkaline-protease was retained about 90% of its activity up to 40°C. The findings suggested that these enzymes providing environmentally compatible processes under relatively mild conditions have potential to be used in several fields such as food processing and detergent industry.

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Balıklıgöl, Türkiye'den İzole Edilen *Bacillus* Suşları Tarafından Üretilen Hidrolitik Enzimlerin Kısmi Karakterizasyonu

ÖZET

Bu çalışmada, Balıklıgöl, Şanlıurfa'dan elde edilmiş ve daha önceden çalışılmamış toprak örneklerinden kırk beş bakteriyel izolat elde edilmiştir. Enzim üretim kapasitelerine göre bu izolatlar arasından BGL-22, BGL-26, BGL-27, BGL-37, BGL-38 ve BGL-39 olarak isimlendirilen altı bakteriyal izolat ileriki denemeler için seçilmiştir. Konvansiyonel ve moleküler tanılama sonuçları, bu bakterilerin *Bacillus* cinsine dahil olduğunu göstermiştir. Bu suşlar arasından *Bacillus* sp. BGL-37, amilaz (11.44 U mL^{-1}), lipaz (1.12 U mL^{-1}) ve proteaz (2.61 U mL^{-1}) açısından en yüksek aktiviteyi göstermiştir. Enzimatik karakterizasyon çalışmaları, alkali proteazın 40°C'ye kadar yaklaşık %90 aktivitesini koruduğunu, asit-kararlı amilaz ve alkali-kararlı lipazın ise 50°C'ye kadar aktivitelerinde herhangi bir düşüş yaşanmadan aktivite gösterdiklerini ortaya koymuştur. Elde edilen bulgular, nispeten ılımlı koşullar altında çevresel olarak uyumlu süreçlerin gerçekleşmesini sağlayan bu enzimlerin, gıdaların işlenmesi ve deterjan endüstrisi gibi çeşitli alanlarda kullanılma potansiyeline sahip olduğunu göstermiştir.

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INTRODUCTION

Enzymes from microbial origin have been mostly preferred due to the countless advantages that they offer as compared to traditional catalysts. Preventing formation of impurities, reducing overall cost of the products, and performing the reactions under mild conditions were among the superiorities resulting from chemo-, regio-, and stereo-selectivity of the enzymes (Sanchez and Demain, 2017; Prasad and Roy, 2018). Although the first industrial enzyme production dates back to the late part of 19th century, the early processes were based on microbial enzymes from *Bacillus licheniformis* and *Aspergillus niger* demonstrated to be safe and thereby indicating the applicability of these enzymes in multifarious food processes (Rastall, 2007;

Ray and Rosell, 2017; Raveendran et al., 2018). Mostly being hydrolytic in action, commercial microbial enzymes serve versatile areas such as food, detergent, leather, agriculture, feed, and textile industries. Thus, the market for industrial enzymes is predicted to reach from $5.01 billion in 2016 to $6.32 billion in 2021 (Chapman et al., 2018). Amylase, lipase and protease constitute the largest market share within industrial enzymes.

For over 500 biotechnological substances, at present, are industrially produced by microbial enzymes. Notwithstanding, some insufficiencies of the enzymes such as low tolerance to solvents or activity lost under extreme temperature and pH conditions restrict the yield of industrial processes which in turn results in an increase in the product cost (Adrio and Demain, 2014; Sanchez and Demain, 2017). Thus, search for the novel enzymes that match specific requirements of the relevant industrial processes will undoubtedly remain an important research topic in the future. Microorganisms, especially bacteria and fungus, are the largest sources of industrial enzymes. Among the bacteria, members of *Bacillus* genus possess varied biochemical pathways and thereby are able to synthesize enzymes as well as other secondary metabolites. High growth rates that shorten production times, extracellular secretion capability of the proteins into the fermentation media, and their long history in industrial use along with the knowledge of their biochemistry and genetics make these bacteria favorable for the synthesis of various biotechnological products (Schallmey et al., 2004; Parrado et al., 2014). Therefore, they continue to be dominant bacterial workhorses in numerous medical, pharmaceutical and agricultural processes.

The aim of this study was to isolate novel *Bacillus* strains capable of producing amylase, lipase and protease with biotechnological importance. To the best of the knowledge, this is the first study investigating the extracellular enzyme production potential of indigenous bacteria from terrestrial areas of Balıklıgöl. Following successive bacterial isolation and screening steps, six bacterial strains identified as *Bacillus* sp. by conventional and molecular identification techniques were selected for comparison of enzyme production. Moreover, partial characterization of the enzymes showing the highest production activities from the best producing strain was performed.

MATERIALS and METHODS

Samples and isolation of bacteria

To select potential extracellular enzyme producing *Bacillus* isolates, soil samples aseptically collected from various locations of Balıklıgöl-Şanlıurfa were exposed to heat treatment at 80°C for 10 min to eliminate vegetative form of any kind of bacterial species. Serially diluted soil samples in sterile 0.85% (w/v) physiological saline solution were spread on Nutrient Agar (NA) plates to allow the germination of remaining spores in order to form vegetative colonies. Morphologically different colonies were chosen and purified by consecutive streaking several times on NA plates. Pure bacterial cultures were stocked in 20% (v/v) glycerol at –80°C.

Screening for enzyme production

Bacterial isolates were scored for enzyme production by spotting onto NA plates including the following components per L of distilled water: peptone, 5.0 g; beef extract, 1.5 g; yeast extract, 1.5 g; NaCl, 5.0 g; agar, 15.0 g. For screening of amylolytic, lipolytic and proteolytic activities, NA plates were supplemented with soluble starch (0.5%, w/v), tributyrin (1%, v/v), and skim milk (10%, w/v), respectively. All plates were incubated at 37°C for 2 days before the measurements of hydrolysis zones. Amylase activities were visualized by flooding with Lugol's solution, comprised of iodine (I_2) and potassium iodide (KI).

Morphological and biochemical identification of bacteria

The isolates were initially subjected to morphological examination as well as Gram and endospore staining. The Gram-positive, spore forming, rod-shaped bacilli were then subjected to additional identification tests. For the genus level identification, biochemical tests were performed as described in Bergey's Manual of Determinative Bacteriology (Bergey and Holt, 1994).

Molecular identification and phylogenetic analysis of bacteria

DNA isolation was performed from pure cultures grown overnight in Luria–Bertani (LB) medium using the PureLink Genomic DNA kit (Invitrogen, USA). By using universal primer pair 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'), 16S rRNA genes were amplified by TrueStart Hot Start Taq DNA polymerase (Thermo Scientific, USA) under the following PCR conditions: DNA polymerase activation at 95°C for 2 min, denaturation at 95°C for 30 sec, annealing at 60°C for 30 sec, and extension at 72°C for 90 sec was repeated for 35 cycles and the reaction was terminated with the final extension at 72°C for 7 min. After which the PCR amplicons were purified, sequencing analysis of the fragments was carried out by ABI 3130XL automated sequencer (Applied Biosystems, USA). Comparisons of the sequences were performed by the BLAST search at the NCBI database. The sequences for 16S rRNA genes of all six newly isolated bacteria were deposited in GenBank under the Accession numbers MH647664 to MH647669.

The evolutionary distances of 16S rRNA regions of the

bacteria were computed using the p-distance method (Nei and Kumar, 2000) and phylogenetic analysis was inferred by using the Neighbor-Joining method (Saitou and Nei, 1987) in MEGA 7 (Kumar et al., 2016). The confidence values were obtained from the bootstrap analysis using 1000 replicates are presented next to the branches (Felsenstein, 1985).

Production of hydrolytic enzymes

The extracellular productions of amylase, lipase and protease were carried out by using submerged fermentation (SmF) in amylase medium (g L^{-1}: soluble starch, 10.0; yeast extract, 3.0; peptone, 5.0; NaCl, 3.0; $MgSO_4.7H_2O$, 0.5) (Deljou and Arezi, 2016); lipase medium (g L^{-1}: tributyrin, 10.0; yeast extract, 5.0; peptone, 5.0; NaCl, 0.5; $CaCl_2$, 0.05; Tween 80, 5.0) (Kumar et al., 2005); and protease medium (g L^{-1}: D-glucose, 10.0; yeast extract, 5.0; peptone, 5.0; K_2HPO_4, 1.0; $MgSO_4.7H_2O$, 0.2) (Mehrotra et al., 1999), respectively. 50 mL of the production media buffered with phosphate buffer (pH 7.0) in Erlenmeyer flasks were inoculated with one mL of overnight cultures of the bacterial strains and incubated at 37°C for 3 days on a rotary shaker at 150 rpm. After the incubation, crude enzyme solutions were obtained by centrifuging the production media at 8000 rpm and 4°C for 10 min.

Enzyme assays

For amylase, the analysis was performed by using the dinitrosalicylic acid (DNS) method (Rick and Stegbauer, 1974). 500 µL of the enzyme solution and 500 µL of 1% soluble starch were incubated at 37°C for 15 min. Then, one mL of DNS reagent was pipetted, and the tube was incubated in boiling water for 5 min. After cooling at room temperature, it was diluted up to 10 mL with distilled water. The absorbance was measured spectrophotometrically in a microplate reader (BioRad iMark, USA) at 546 nm. A unit of amylase was defined as the amount of enzyme catalyzing the hydrolysis of soluble starch to one µmol of maltose per mL per min under assay conditions.

The lipase activity was analyzed by quantifying the release of p-nitrophenol from p-nitrophenyl palmitate (pNPP) according to the slightly modified method by Rapp and Backhaus (1992). The enzyme solution (500 µL) was added to 750 µL pNPP solution and incubated at 37°C for 15 min. By adding 100 µL of 1 M sodium carbonate (Na_2CO_3) into the tube, the reaction was terminated and then re-incubated at 37°C for 15 min. The absorbance of the filtrate was measured at 415 nm using a microplate reader. A unit of lipase activity was expressed as the amount of the enzyme that releases one µmol of p-nitrophenol from pNPP per mL per min under assay conditions.

The protease activity was determined using Sigma's universal protease assay with minor modifications. 320 µL of the enzyme solution was mixed with 800 µL of 0.65% casein solution. After 15 min incubation at 37°C, the reaction was terminated by pipetting 800 µL of 0.11 M trichloroacetic acid (TCA) solution. The tube was allowed to stand at 37°C for 30 min. Then, 500 µL of the filtrate obtained by centrifuging at 8000 rpm, 1.25 mL of 0.5 M Na_2CO_3 solution and 250 µL of Folin-Ciocalteu reagent (1:4 dilution) were mixed, and the mixture was incubated at 37°C for 30 min. The absorbance was read at 655 nm by a microplate reader. A unit of protease activity was expressed as the amount of the enzyme that catalyzes the hydrolysis of casein to one µmol of tyrosine per mL per min under assay conditions.

Estimation of protein content

Protein concentrations in the crude extracts were quantified by using Bradford reagent (Bradford, 1976) and bovine serum albumin was used as a standard.

Effects of pH and temperature on enzyme activity and stability

The optimum pH values for the enzymes were determined with soluble starch, casein and pNPP as substrates, dissolved in 50 mM of different buffers (citrate-phosphate, pH 5.0 and 6.0; phosphate, pH 7.0 and 8.0; glycine-NaOH, pH 9.0 and 10.0). The effect of pH on stability was investigated by incubating the enzyme solution without substrate at varied pH conditions ranging from 3.0 to 10.0 adjusted with 0.2 M HCl or 1 M NaOH for 2 h at 37°C.

The effect of temperature on enzyme activity was evaluated by performing the analysis procedure within a temperature range of 30-60°C in 50 mM phosphate buffer (pH 8.0) containing substrate solutions as described above. Thermostability was assessed by incubating the enzyme solution at different temperatures varied from 20 to 70°C for 2 h.

Statistical analysis

All tests in the study were analyzed triple and the findings were presented as the mean of three independent experiments.

RESULTS AND DISCUSSION

Screening of bacteria producing hydrolytic enzymes

For evaluation of enzyme production, forty-five bacteria were isolated from soil samples collected from Balıklıgöl. Preliminary screening for amylase, lipase and protease were carried out on agar plates including starch, tributyrin and skim milk, respectively. Among the isolates, the bacteria designated as BGL-39 (17 mm), BGL-26 (13 mm) and BGL-37 (27 mm) exhibited the highest clearing zones for amylase, lipase and protease, respectively (Table 1).

Table 1. Hydrolysis zones formed on the agar plates including starch, tributyrin and skim milk individually for screening of hydrolytic enzymes

Çizelge 1. Hidrolitik enzimlerin taranması için nişasta, tributirin ve yağsız sütü ayrı ayrı içeren agar petrilerinde oluşan hidroliz zonları

Strain no.	Hydrolysis zones (mm)		
	Amylase	Lipase	Protease
BGL-22	2	10	20
BGL-26	6	13	24
BGL-27	14	4	25
BGL-37	12	9	27
BGL-38	8	9	14
BGL-39	17	2	14

The isolates showing an ability to produce all three enzymes, including strains BGL-26, BGL-37 and BGL-39, were selected for conventional and molecular identification studies.

Phenotypic characterization of potential enzyme-producing bacteria

Morphological identification results demonstrated that all isolates were Gram-positive, spore forming rod shaped bacteria. Biochemical tests including motility, citrate hydrolysis, Voges-Proskauer (VP), Methyl Red (MR), indole production, oxidase, urease, catalase, starch and gelatin hydrolysis, nitrate reduction, glucose fermentation and production of H_2S were performed for genus level identification of the bacterial isolates. The results were summarized in Table 2. Although some biochemical test results were different among the bacterial isolates, it was determined that the isolates belonged to the *Bacillus* genus.

Table 2. Morphological and biochemical characteristics of bacteria

Çizelge 2. Bakterilerin morfolojik ve biyokimyasal karakteristikleri

Characteristics	Strain no.					
	BGL-22	BGL-26	BGL-27	BGL-37	BGL-38	BGL-39
Gram's reaction	+	+	+	+	+	+
Morphology	Rod	Rod	Rod	Rod	Rod	Rod
Spore formation	+	+	+	+	+	+
Motility	+	+	+	+	+	+
Indole production	–	–	–	–	–	–
Methyl red	–	–	–	–	+	+
Voges-Proskauer	+	+	+	+	+	+
Citrate utilization	+	–	–	–	–	–
Catalase	+	+	+	+	+	+
Oxidase	–	–	–	–	–	+
Urease	–	–	–	–	–	–
H_2S production	–	–	–	–	–	–
Starch hydrolysis	+	+	+	+	+	+
Gelatin hydrolysis	+	+	+	+	+	+
Glucose fermentation	+	+	+	+	+	+

Molecular identification and phylogenetic analysis of bacteria

To gain further insight on the bacterial identification, a molecular technique whose reliability is more than that of conventional identification, was also used. 16S rRNA regions of the isolates were amplified using 27F/1492R primer pair and the amplification yielded a single PCR product ranging from 1429 to 1392 bp. The BLASTN tool-based assignment of all isolates against the NCBI database found out that the selected bacteria belonged to the *Bacillus* genus with 100% homology. Phylogenetic tree constructed with Neighbor-Joining method also showed that newly isolated *Bacillus* strains share close relationships with other members of *Bacillus* genus (Figure 1). Despite occasional inconsistencies between conventional and molecular identification techniques, the identification results we obtained supported each other.

Production and characterization of hydrolytic enzymes

Extracellular production of the enzymes was investigated using SmF in media buffered at pH 7.0 and the findings were presented in Table 3. Briefly, the enzymes from strain BGL-37 showed the highest activities for the production conditions tested. On the other hand, for amylase and protease, the highest specific activities were obtained by strains BGL-38 (82.00 U mg^{-1}) and BGL-27 (23.27 U mg^{-1}) respectively due to the low amounts of total proteins expressed. Compared to the findings with other results in the literature, we found that strain BGL-37 was a good producer of the hydrolytic enzymes (Divakaran et al., 2011; Jamrath et al., 2012; Sharma et al., 2014) and therefore, the enzymes from this strain were selected for further studies for determining the influences of pH and temperature on activity and stability.

The optimal pH and temperature conditions for strain BGL-37 amylase were pH 7.0 and 37°C (Figure 2). The enzyme displayed the highest stability at acidic pHs, followed by neutral and alkaline values. The enzyme showed over 95% of its relative activity for 2 h at 37°C in acidic conditions while it was found to be stable approximately 80% of the activity at alkaline pH values (Figure 2a). The enzyme remained nearly 100% stable up to 50°C. After that temperature, the enzyme activity rapidly decreased. However, the enzyme still showed 66% of its activity at 70°C (Figure 2b).

Compared to the stability of strain BGL-37 amylase with results in literature, we observed that amylase from this study had greater pH and temperature stability (Mahdavi et al., 2010; Dahiya and Rathi,

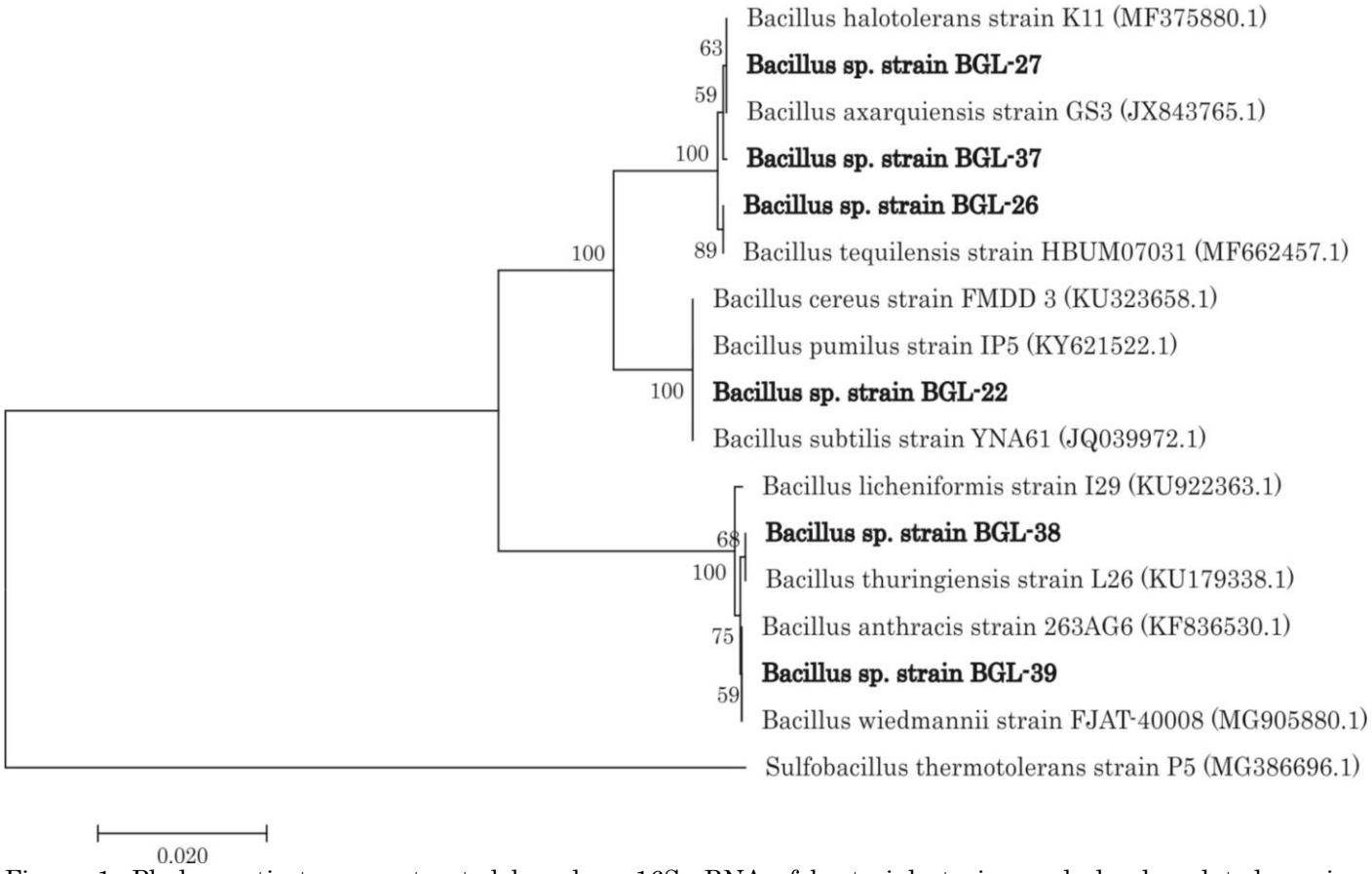

Figure 1. Phylogenetic tree constructed based on 16S rRNA of bacterial strains and closely related species. *Sulfobacillus thermotolerans* was used an out-group taxon. Bootstrap percentages are shown at branch points

Şekil 1. Bakteri suşları ve yakın türlerin 16S rRNA'larına göre oluşturulmuş filogenetik ağaç. Out-group takson olarak Sulfobacillus thermotolerans kullanılmıştır. Bootstrap yüzdeleri, dal noktalarında gösterilmiştir

Table 3. Extracellular production of hydrolytic enzymes by *Bacillus* sp. strains (values represent mean of three experimental results)

Çizelge 3. Bacillus sp. suşları tarafından hidrolitik enzimlerin hücre dışı üretimi (değerler üç deneysel sonucun ortalamasını temsil etmektedir)

Strain no.	Enzymes								
	Amylase			Lipase			Protease		
	Activity (U mL^{-1})	Total protein (mg mL^{-1})	Specific activity) (U mg^{-1})	Activity (U mL^{-1})	Total protein (mg mL^{-1})	Specific activity (U mg^{-1})	Activity (U mL^{-1})	Total protein (mg mL^{-1})	Specific activity (U mg^{-1})
BGL-22	0.92	0.05	18.40	0.16	0.08	2.00	2.13	0.18	11.83
BGL-26	11.13	0.23	48.39	0.58	0.10	5.80	1.97	0.27	7.30
BGL-27	6.17	0.18	34.28	1.04	0.17	6.12	2.56	0.11	23.27
BGL-37	11.44	0.14	81.71	1.12	0.11	10.18	2.61	0.12	21.75
BGL-38	3.28	0.04	82.00	0.24	0.11	2.18	0.67	0.12	5.58
BGL-39	1.61	0.13	12.38	0.54	0.10	5.40	0.67	0.03	22.33

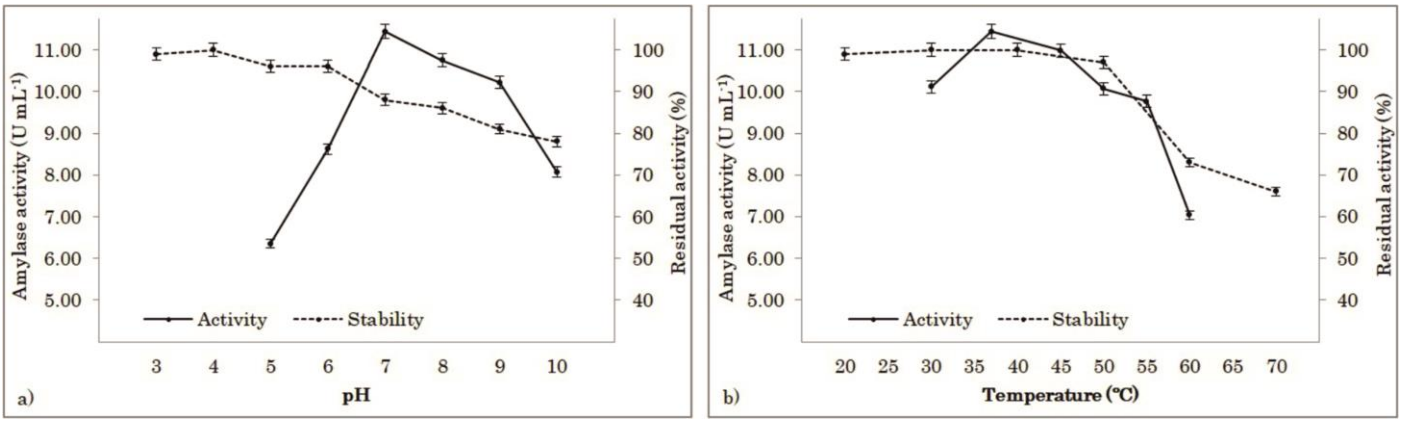

Figure 2. Effects of pH (a) and temperature (b) on activity and stability of amylase produced by strain BGL-37. Bars represent standard errors of means, n=3

Şekil 2. pH (a) ve sıcaklığın (b) BGL-37 suşu tarafından üretilen amilaz aktivitesi ve stabilitesi üzerine etkileri. Barlar standart hataların ortalamalarını temsil etmektedir, n=3

2015). Mahdavi et al. (2010) aimed to characterize an α-amylase from acid-neutralizing *Bacillus cereus* and investigated its thermal stability. Although the enzyme remained stable more than 80% at 60°C for 1 h, complete inactivation was detected after incubation at 70°C for 25 min. In another study, Dahiya and Rathi (2015) studied an alkaline amylase for application as a detergent additive from *B. licheniformis* MTCC1483. The researchers indicated that about 80% of the original activity of the amylase remained relatively stable for 45 min at 40°C at varied pH values from 6.0 to 10.0. However, the enzyme lost nearly 50% of its relative activity at pH 10.0 for 1 h. As a result of thermo-acid-stable properties, amylase from strain BGL-37 has potential usage in baking, fruit juice processing and starch liquefaction.

Lipase from strain BGL-37 showed activity at the pH values ranging from 5.0 to 10.0, and the optimal activity was observed in alkaline condition at pH 8.0 (Figure 3a). Further increasing or decreasing in the pH value of the solution reduced the enzyme activity dramatically. The enzyme remained stable 69% at pH 3.0, and its stability increased as pH value increased. It was found to be stable 100% at pH 10.0. BGL-37 lipase was optimally active at 37°C (Figure 3b). Below or above this temperature, the activity rapidly decreased. As stated for strain BGL-37 amylase, the lipase enzyme retained 100% of its activity up to 50°C and its relative stability decreased with increasing temperature. Even so, it was stable more than 60% at 70°C for 2 h. Compared to other lipases in literature, strain BGL-37 lipase showed higher pH and temperature stability. For instance, in a study, Saraswat et al. (2017) investigated a thermotolerant alkaline lipase from *Bacillus* sp. and found that the lipase remained stable more than 85% of its relative activity at pH 8.0 while over 60% at pH 6.0, 7.0 and 9.0. According to these results, the researchers indicated that this enzyme could be used in the leather industry and detergent formulation. The authors also studied the thermostability of the enzyme, and 40% of the lipase activity was observed at 50°C, while this rate decreased to about 20% at 55 and 60°C.

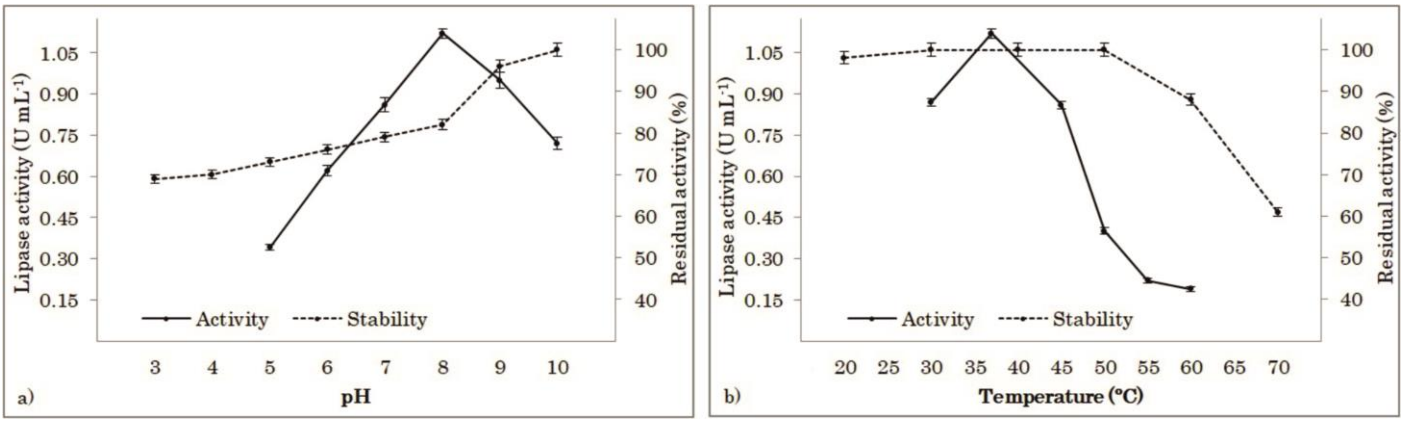

Figure 3. Effects of pH (a) and temperature (b) on activity and stability of lipase produced by strain BGL-37. Bars represent standard errors of means, n=3

Şekil 3. pH (a) ve sıcaklığın (b) BGL-37 suşu tarafından üretilen lipaz aktivitesi ve stabilitesi üzerine etkileri. Barlar standart hataların ortalamalarını temsil etmektedir, n=3

In another study, Sharma et al. (2002) studied an alkaline and thermostable lipase from *Bacillus* sp. RSJ-1 and revealed that the enzyme remained stable nearly 40% of its relative activity at 70°C for 2 h. Considering the lipase activity of strain BGL-37 in a wide range of pH and temperature, the enzyme might have a potential application in enhancement of cheese ripening or enzyme modified cheese processes. Protease from strain BGL-37 showed its highest activity at pH 8.0, followed by at pH 7.0 and 9.0 (Figure 4a). Unlike many other proteases studied before, the enzyme was able to preserve over 90% of its activity at the pH conditions tested. The protease showed its maximal activity at 37°C followed by at 45 and 30°C and remained stable nearly 90% up to 40°C. Above this temperature, the stability decreased sharply and 31% of the activity was observed at 70°C for 2 h (Figure 4b). In several studies, researchers obtained alkaline and thermotolerant proteases from different *Bacillus* strains and investigated their pH and thermal stability (El Hadj-Ali et al., 2007; Bhunia et al., 2011; Gomaa, 2013; Yilmaz et al., 2016). The stability of BGL-37 protease was at a comparable level to those of above-mentioned enzymes, even in some cases protease from this study had higher pH stability and thermostability. For example, the alkaline protease from *Bacillus pumilus* ATCC7061 retained about 80% of its activity at pH 8.0 and 9.0 for 1 h at 40°C (Gomaa, 2013) although protease from strain BGL-37 remained stable over 90% at all tested pH values. In the study conducted by El Hadj-Ali et al. (2007), a detergent stable protease from *B. licheniformis* NH1 showed no activity at 70°C regardless of presence or absence of $CaCl_2$, while strain BGL-37 protease remained stable more than 30% of its activity. Consequently, strain BGL-37 protease might be used as flavor enhancing agent in food products due to its alkali tolerance.

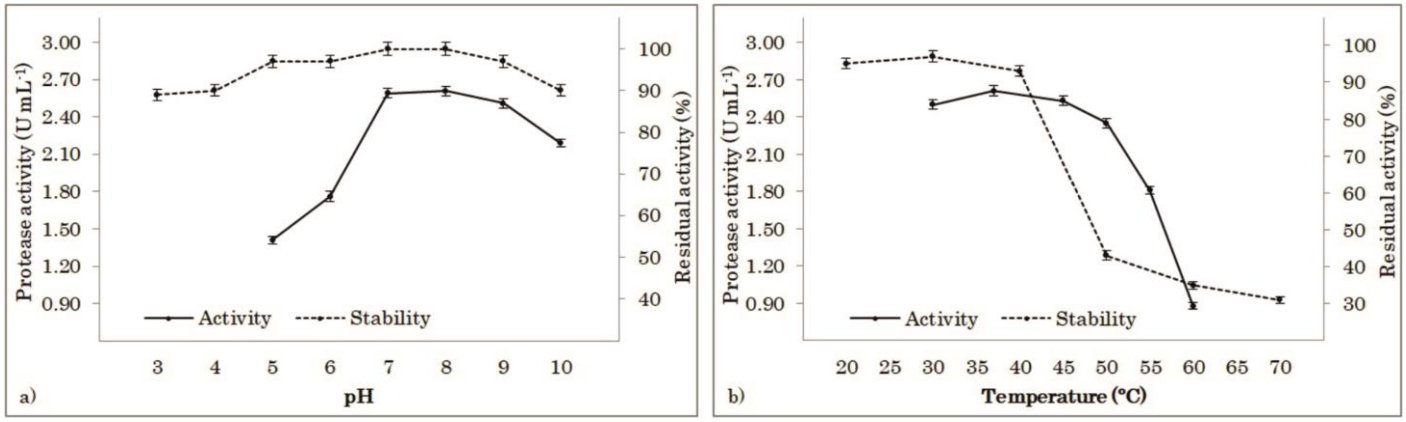

Figure 4. Effects of pH (a) and temperature (b) on activity and stability of protease produced by strain BGL-37. Bars represent standard errors of means, n=3

Şekil 4. pH (a) ve sıcaklığın (b) BGL-37 suşu tarafından üretilen proteaz aktivitesi ve stabilitesi üzerine etkileri. Barlar standart hataların ortalamalarını temsil etmektedir, n=3

CONCLUSIONS

Industrial enzyme market will continue to grow steadily for improved outcomes such as environmental sustainability and efficiencies in biotechnological processes. Needless to say, interest in microorganisms capable of producing compatible enzymes that can meet all the requirements of a specific biotechnological process will increase. The majority of the enzymes produced by biotechnology companies over the world are originated from *Bacillus* species. Thus, we focused on the *Bacillus* members from the local environments to evaluate the production potentials for extracellular enzymes including amylase, lipase and protease. To isolate novel *Bacillus* species, we selected terrestrial areas of Balıklıgöl which has not been studied before. Partial characterization studies indicating that all three enzymes produced by the novel *Bacillus* strains were alkali-tolerant and fully functional at 40°C suggest that these enzymes are good prospect for their potential substitution especially in food and detergent industries. When considering the ease of genetic manipulation, *Bacillus* sp. BGL-37 might be used as a source for evaluation of possible modification of these enzymes offering a better alternative in different greener industrial processes not only by energy saving but also reducing hazardous substances/wastes.

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Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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