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Determination of Total Xylanase Activities of Various Thermophilic Bacteria

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ABSTRACT: Enzymes, which have important metabolic functions in living organisms, make important contributions to human beings by using them for different purposes in many areas such as economy, food, agriculture, and industry as part of the daily lives. Today, enzymes, whose production and usage purposes, are increasing, were generally obtained from plant, animal, and microorganisms. In this study; the enzyme producing isolates and their total xylanase activities of various thermophilic bacteria (*Bacillus coagulans*, *Bacillus licheniformis*, *Bacillus subtilis*, *Bacillus* sp. and *Geobacillus kaustophilus*) isolated from previously hot springs in Eastern and Southeastern Anatolia regions were determined. In the enzyme activity of the isolates, 47 isolates showed xylanase activity less than 0.1 U/ml, 31 isolates were between 0.1 U/ml and 0.2 U/ml. However, 5 isolates of *B. subtilis* [BTX3 (0.206 U/ml), BTX6 (0.286 U/ml), BTX22 (0.2 U/ml), BTX27 (0.203 U/ml) and BTX32 (0.206 U/ml)] were exhibited highest activity for xylanase enzyme production.

Keywords: *Bacillus subtilis*, xylanase, enzyme activity, hot springs, thermophilic bacteria

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INTRODUCTION

The bacteria are a unique group of microorganisms that thrive under extreme environmental conditions such as high/low temperature that most life forms difficult to survive in. It is thus not surprising that these microorganisms have developed important strategies and mechanisms to survive in different temperature conditions. In this context, the bacteria are categorized as psychrophiles, mesophile, thermophiles and hyperthermophiles according to temperature requirements. (Baltaci et al., 2017). Thermophilic bacteria are commonly found in soil, hot spring sources and volcanic habitats (Zeikus, 1979). They, capable of optimal reproduction at a growing temperature above 60°C, are more resistant to both stable protein structures and various chemicals (Madigan et al., 2006). Thus, the thermophilic bacteria and their enzymes are often used in many industrial areas.

Commercial enzyme production is a popular research area because of their faster reproduction, easy production, ability to reproduce in extreme conditions, alternative to chemicals and cell structures is a very popular in study by scientists (Aehle, 2007; Hubbe, 2016). The usage of enzyme as biocatalysts in industrial processes protects both nature and human beings from the costs and harms of many toxic compounds and solvents. In general, mesophilic enzymes are used in some studies that can cause various degradation such as substrate solubility, low viscosity, disruption of high temperature steps and the risk of contamination (Demarche et al., 2012; Kiran et al., 2006). However, the use of enzymes that are active at high temperatures compared to mesophilic ones are more compatible in pre-processing and reduces the cost by eliminating the need for cooling processes required for enzymatic processes. The usage of thermophilic enzymes increase interest in industrial processes because of their activity and stability at high temperatures and lead to the development of a biologically based economy.

Cellulose, hemicellulose and lignin are the key components of plant cell walls, and at the same time these components have the potential to be converted into useful final products such as bioethanol, xylitol, and other simple sugars (Ding et al., 2018; Kulkarni et al., 1999; Subramaniyan and Prema, 2002). Hemicellulosic structures are the secondary component derived from lignocellulosic agricultural residues. Hemicellulose forms over 30% of the dry weight of terrestrial plants and consist mainly of xylans used in the production of xylooligosaccharides (Krengel and Dijkstra, 1996). Xylans are polysaccharides composed of β-1,4-coupled xylopyranose units. One of the most important enzymes that can hydrolyze this structure is the 1,4-β-endo xylanase (EC 3.2.1.8) enzymes. Xylans, one of the main components of plant cell walls, especially constitute the main food source of farm animals. In addition, they are the raw material of industrial products encountered in baking bread making, paper pulp making and bleaching processes in the industrial and food sector. Therefore nowadays, the use of microbial enzymes that degrade xylans has become very popular and the thermophilic xylanase enzyme has also a highly commercially important position at this point (Drout et al., 2019).

In this study, it was aimed to determine the 1,4-β-endo xylanase enzyme activities of 83 thermophilic bacteria obtained from some Eastern and Southeastern Anatolia hot springs (Pasinler, Hasanabdal, Hista, Diyadin, Davut, Köprü, Dargecit and Guclukonak), that were isolated and identified in previous studies (Ulucay et al., 2021). Thus, the xylanases obtained from these thermophilic microorganisms will be have the opportunity to be used in industrial processes and fields that are carried out in high temperature and pH processes with further studies.

MATERIAL AND METHODS

Bacterial Isolates

The bacteria isolated and identified earlier from thermal hot springs in the Eastern and Southeastern provinces of Turkey including Agri (Diyadin, Davut, and Kopru), Erzurum (Pasinler), Van

(Hasanabdal), Siirt (Hista), Mardin (Dargecit) and Sirnak (Guclukonak) were used in this study (Ulucay, 2018; Ulucay et al., 2021).

Determination of Enzyme Activities

All of the isolates were streaked on an agar medium containing 10 g xylan, 2 g tryptone, 15 g agar, 2 g yeast extract, 2 g KH_2PO_4, 0.5 g $MgSO_47H_2O$ and the xylanase activity of the isolates that form a transparent zone on the agar plate was recorded as positive (Ding et al., 2004).

Enzyme Assays

Xylanase activities of isolates were determined spectrophotometrically by measuring the number of water-soluble compounds as a result of the hydrolysis of azurine cross-linked birch xylan (Megazyme, Ireland). The liquid LB medium containing xylan was inoculated with fresh culture at pH 7.0, and cultures were developed for 24 hours at 55°C, 250 rpm. Bacterial isolates were centrifuged at +4ºC and 6000 rpm for 20 minutes. The culture supernatant precipitated by the addition of v/v cold ethanol (96%) at -20ºC overnight and then, it was centrifuged at +4°C and 8000 rpm for 20 minutes. The enzyme precipitate collected at the bottom was dissolved in sodium phosphate buffer (0.1 M, pH 6.8) and it was obtained a total enzyme solution of 250 ml (Srivastava et al., 1987). According to dinitro salicylic acid (DNS) method was used to determine enzyme activity (Miller GL, 1959). The assay mixture containing 0.9 ml substrate solution and 0.1 ml suitably diluted enzyme solution with oat spelt xylan 1% (w/v) in the Hungate tube was incubated at 65°C for 1 hour and the reaction was stopped by addition of 1 ml DNS reagent. Then, the reaction was boiled for 5 min, and when the mixture was reached room temperature, the absorption was measured at 540 nm with spectrophotometer (ACTG Gene UVS-99). One unit (U) of xylanase activity was defined as the amount of enzyme that catalyzes the release of 1 umol of the xylose equivalent per minute (Ding et al., 2018).

RESULTS AND DISCUSSION

In this study, firstly, the xylanase enzyme activities of the isolates were determined and it was seen that all isolates had xylanase activity (Table 1). The bacterial isolates exhibiting xylanase activity grew optimally at 60^{o}C and pH 7. Therefore, these isolates were evaluated as thermophile xylanases (Ulucay et al., 2021).

The enzyme activity results of the isolates have been presented in Fig.1. As seen in Fig.1, 47 isolates were exhibited xylanase activity less than 0.1 U/ml, 31 isolates were between 0.1 U/ml and 0.2 U/ml, 5 isolates [BTX3 (0.206 U/ml), BTX6 (0.286 U/ml), BTX22 (0.2 U/ml), BTX27 (0.203 U/ml) and BTX32 (0.206 U/ml] were greater than 0.2 U/ml.

Table 1 The bacterial isolates used for assay of enzyme activities (Ulucay et al., 2021)

Isolate Code	Bacterial Isolates
BTX1, BTX2, BTX3, BTX4, BTX5, BTX6, BTX7, BTX8, BTX9, BTX10, BTX11, BTX12, BTX13, BTX14, BTX15, BTX22, BTX23, BTX24, BTX25, BTX26, BTX27, BTX28, BTX30, BTX31, BTX32, BTX33, BTX34, BTX35, BTX48, BTX60, BTX61, BTX81, BTX78	*Bacillus subtilis*
BTX16, BTX17, BTX18, BTX19, BTX20, BTX21, BTX29, BTX36, BTX37, BTX38, BTX39, BTX40, BTX41, BTX82	*Bacillus licheniformis*
BTX42, BTX43, BTX44, BTX45, BTX46, BTX47, BTX49, BTX50, BTX51, BTX52, BTX69, BTX70, BTX71, BTX77, BTX80	*Geobacillus kaustophilus*
BTX53, BTX54, BTX55, BTX56, BTX57, BTX58, BTX59, BTX72, BTX73, BTX79	*Bacillus* sp.
BTX62, BTX63, BTX64, BTX65, BTX66, BTX67, BTX68, BTX74, BTX75, BTX76, BTX83	*Bacillus coagulans*

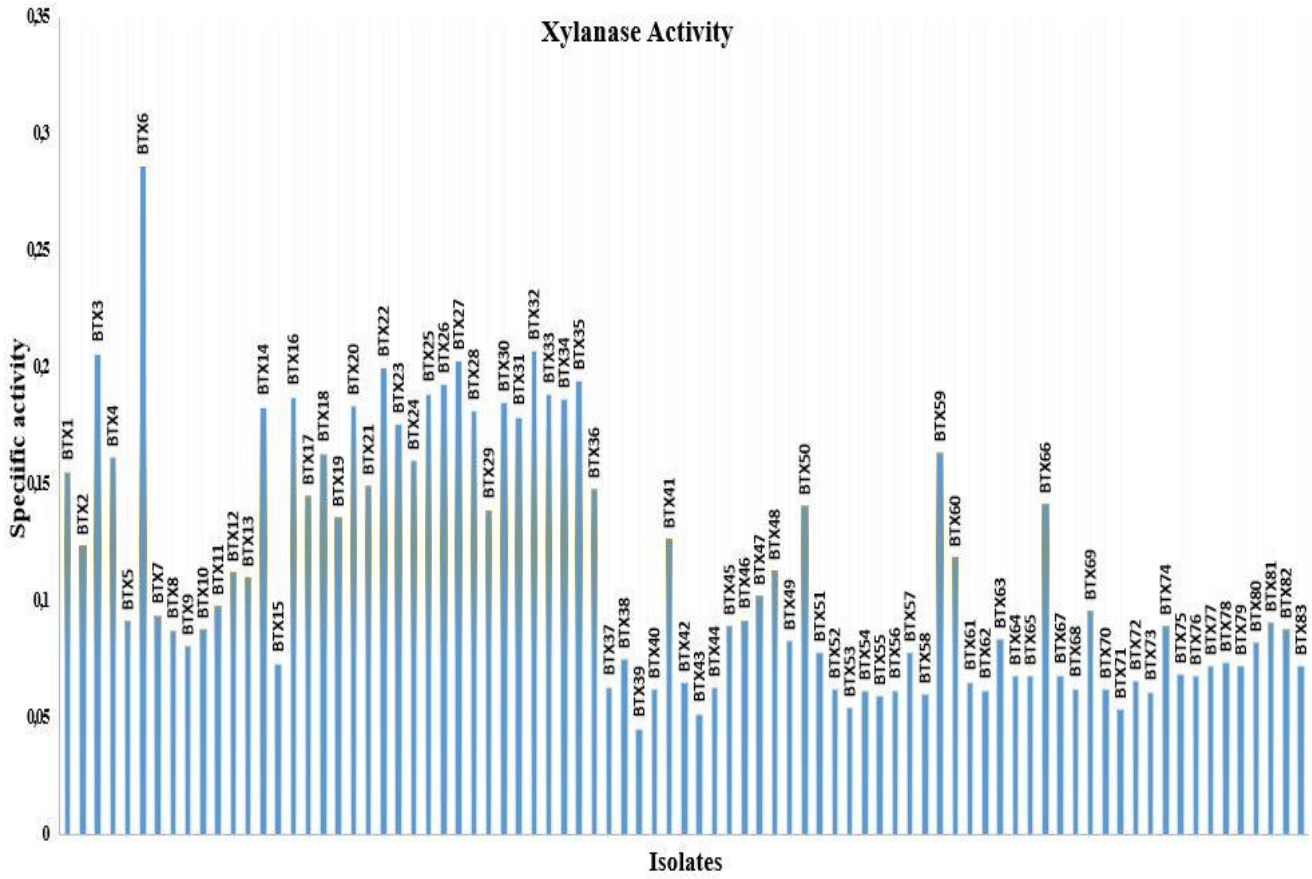

Figure 1 Total xylanase activities of isolates

Microbial enzymes are in a very important position in terms of being resistant to extreme (such as high temperature) conditions. Enzymes obtained from thermophilic microorganisms are widely used in fields such as industry. Because extremophile organisms are not affected by the pH, temperature and other negative factors of the environment, the stability and activities of the enzymes produced by them are also very high. Thermophilic microorganisms have the ability to reproduce under extreme conditions. Therefore, they can produce a wide variety of usable products even under unfavorable conditions. Many enzymes such as cellulase, xylanase, catalase, lactase, lipase, sucrase, pullunase, pectinase, amylase, and protease are obtained recombinantly from thermophilic microorganisms and are widely used in the industry. These stable and durable enzymes are widely used in many fields such as textile, food, detergent, bread dough, paper bleaching, beverage industry and health (pharmaceutical) (Niehaus et al., 1999). The high temperature values of the hot springs in the regions discussed in this study also make the enzymes to be preferred at this point important. Therefore, in the study; the focus has been on the xylanase enzyme, which is frequently used and sought in the industry, and higher efficiency xylanases that can be used commercially in the market have been determined.

Bacillus species are a common microorganism that can be isolated from many environments, including hot springs. Especially considering the enzyme stability and growth performance in extreme conditions, they attract the attention of researchers in enzyme production. Besides these enzymes obtained from *Bacillus* spp. meet the enzyme needs in many industrial fields such as starch, pastry, bread, detergent, fruit juice, paper bleaching, textile and beer making (Niehaus et al., 1999). Although some fungi are used in the production of xylanase, which is one of the most important enzymes widely used in the industry, it is known that bacteria, especially *Bacillus* species, are of great importance (de Sousa Gomes et al., 2017). It is known that many *Bacillus* species such as *B. cereus* (Roy and Habib,

2009), *Bacillus* sp. (Hiremath and Patil, 2011), *B. subtilis*, *B. licheniformis* and *Geobacillus thermodenitrificans* produce xylanase (Guo et al., 2012). *B. subtilis* is one of the most preferred thermophilic *Bacillus* species for the commercially produced xylanase enzyme (Banka et al., 2014). Haddar et al. (2012) stated the xylanase activity as 7.23 IU/mL under optimized conditions from a Bacillus they isolated. Besides, Irbe et al. (2014) determined the highest xylanase activity from a mold they isolated as 9.4 IU/mL (Haddar et al., 2012; Irbe et al., 2014). The isolated microorganism (*Bacillus* spp.) showed the lowest activity on the tested agricultural wastes as 25 IU/mL on the corncob. In addition, it has xylanase activity in the range of 33-37 IU/mL with other agricultural wastes (Kocabas vd. (2017). Ammoneh et al. (2014) reported that optimum pH and temperature values for xylanase activities were 7.0 and 55ºC for three *bacilli* namely, SY30A, 6.0 and 60ºC for SY185C and SY190E, and specific activities were 1157, 915 and 794 U/g for SY30A, SY185C and SY190E xylanases, respectively (Ammoneh et al., 2014). Guler F. (2020) Isolates showing high xylanase activity (*Bacillus*), isolate no. 39 and 67 showed xylanase activity of 13.33 and 5.57 U/mL, respectively (Guler 2020). In parallel with the information given in the literature, the specific enzyme activity of isolates determined that 47 isolates were less than 0.1 U/ml, 31 isolates were between 0.1 - 0.2 U/ml, and 5 isolates were less than 0.2 U/ml.

It has been reported that the bacteria showed optimum activity in different pH ranges by many researchers. For example, Dusterhoft et al. (1997) reported that the xylanase enzyme got from *Sulfolobus solfataricus* at pH 7.0 has optimum activity (Düsterhöft et al., 1997); Wainø and Ingvorsen (2003) reported that the optimum activity value of the xylanase enzyme got from *Humicola insolens* is between pH 6.0-6.5 (Wainø and Ingvorsen, 2003); Cannio et al. (2004) reported that the optimum activity of the xylanase enzyme got from the *Halorhabdus utahensis* isolate is at pH 7.0 (Cannio et al., 2004); Kumar et al. (2004) stated that the optimum activity of *Bacillus* living in an alkaline environment is pH 8.0 (Kumar et al., 2004); Annamalai et al. (2009) reported that the xylanase isolated from *B. subtilis* they isolated from the river water edge had the highest enzyme activity in the pH range 7.0-10.0 (Annamalai et al., 2009). At the same time some *Bacillus* species have also been reported in the literature with optimum activity in different pH ranges (5.0, 5.5, 5.6, 6.0, 6.5 and 7.0) (Avcioglu et al., 2005; Gallardo et al., 2004). Guler FM (2020) determined the optimum pH as 8 in two separate samples in study (Guler 2020).

In the literature, it has been observed that the activity of the xylanase enzyme was in different temperature scales. Enzymes are required in some processes at high temperatures, and in some studies that require these enzymes to be active at these high temperatures, heat treatment is desired. For this reason, enzymes active in thermophilic environment have been preferred more than mesophilic ones. As the use of enzymes instead of chemical catalysts in the industry becomes widespread, the damage to the environment has decreased significantly and enzymes have become a very important ecological alternative at this point. As thermophilic enzymes continue to be discovered, they will be used in more processes as biocatalysts, and their effects will be seen both environmentally and economically in the long term.

CONCLUSIONS

Bacillus spp. are known to be an important xylanase producer, especially *B. cereus*, *B. subtilis* and *B. licheniformis*, in previous studies. Therefore, it has been determined to enzyme activities of various thermophilic isolates isolated from different hot springs in this study. It has been found that *B. subtilis* exhibited highest activity for xylanase enzyme production. At the same time, it is thought to the enzyme

had the ideal pH property that would easily adapt to the feed industry, paper industry, bread making, and other fields.

Conflict of Interest

The authors declare that they have contributed equally to the article.

Author's Contributions

The article authors declare that there is no conflict of interest between them.

REFERENCES

Aehle W, 2007. Enzymes in industry: production and applications: John Wiley, Sons.

Ammoneh H, Harba M, Akeed Y, Al-Halabi M, Bakri Y, 2014. Isolation and identification of local *Bacillus* isolates for xylanase biosynthesis. Iranian journal of microbiology, 62, 127-132.

Annamalai N, Thavasi R, Jayalakshmi S, Balasubramanian T, 2009. Thermostable and alkaline tolerant xylanase production by *Bacillus subtilis* isolated form marine environment. Indian Journal of Biotechnology 8(3):291-297

Avcioglu B, Eyupoglu B, Bakir U, 2005. Production and characterization of xylanases of a *Bacillus* strain isolated from soil. World Journal of Microbiology and Biotechnology, 211, 65-68.

Baltaci MO, Genc B, Arslan S, Adiguzel G, Adiguzel A, 2017. Isolation and Characterization of Thermophilic Bacteria from Geothermal Areas in Turkey and Preliminary Research on Biotechnologically Important Enzyme Production. Geomicrobiology Journal, 341, 53-62.

Banka AL, Albayrak Guralp S, Gulari E, 2014. Secretory Expression and Characterization of Two Hemicellulases, Xylanase, and β-Xylosidase, Isolated from *Bacillus Subtilis* M015. Applied Biochemistry and Biotechnology, 1748, 2702-2710.

Cannio R, Di Prizito N, Rossi M, Morana A, 2004. A xylan-degrading strain of *Sulfolobus solfataricus*: isolation and characterization of the xylanase activity. Extremophiles, 82, 117-124.

De Sousa Gomes K, Maitan-Alfenas GP, de Andrade LGA, Falkoski DL, Guimarães VM, Alfenas AC, de Rezende ST, 2017. Purification and characterization of xylanases from the fungus *Chrysoporthe cubensis* for production of xylooligosaccharides and fermentable sugars. Applied biochemistry and biotechnology, 1822, 818-830.

Demarche P, Junghanns C, Nair RR, Agathos SN, 2012. Harnessing the power of enzymes for environmental stewardship. Biotechnology Advances, 305, 933-953.

Ding C, Li M, Hu Y, 2018. High-activity production of xylanase by *Pichia stipitis*: purification, characterization, kinetic evaluation and xylooligosaccharides production. International journal of biological macromolecules, 117, 72-77.

Ding CH, Jiang ZQ, Li XT, Li LT, Kusakabe I, 2004. High activity xylanase production by *Streptomyces olivaceoviridis* E-86. World Journal of Microbiology and Biotechnology, 201, 7-10.

Drout RJ, Robison L, Farha OK, 2019. Catalytic applications of enzymes encapsulated in metal–organic frameworks. Coordination Chemistry Reviews, 381, 151-160.

Dusterhoft EM, Linssen VAJM, Voragen AGJ, Beldman G, 1997. Purification, characterization, and properties of two xylanases from *Humicola insolens*. Enzyme and Microbial Technology, 206, 437-445.

Gallardo O, Diaz P, Pastor FI, 2004. Cloning and characterization of xylanase A from the strain *Bacillus* sp. BP-7: comparison with alkaline pI-low molecular weight xylanases of family 11. Current Microbiology, 484, 276-279.

Guler F, 2020. Optimization of Xylanase Production from Diverse Agricultural Wastes by Indigenous Isolates of *Bacillus* Species. Ankara University Graduate School of Natural and Applied Sciences, Doctoral Thesis (Printed).

Guo G, Liu Z, Xu J, Liu J, Dai X, Xie D, Zheng K, 2012. Purification and characterization of a xylanase from *Bacillus subtilis* isolated from the degumming line. Journal of basic microbiology, 524, 419-428.

Haddar A, Driss D, Frikha F, Ellouz-Chaabouni S, & Nasri M, 2012. Alkaline xylanases from *Bacillus mojavensis* A21: production and generation of xylooligosaccharides. International Journal of Biological Macromolecules, 514, 647-656.

Hiremath Ks, Patil Cs, 2011. Isolation, production and characterization of alkali thermostable xylanase from newly isolated Bacillus sp. International Journal of Biotechnology Applications, 3, 48-51.

Hubbe MA, 2016. Catalysts inspired by life. Biofuel Research Journal, 33, 430.

Irbe I, Elisashvili V, Asatiani MD, Janberga A, Andersone I, Andersons B, Grinins, J, 2014. Lignocellulolytic activity of Coniophora puteana and Trametes versicolor in fermentation of wheat bran and decay of hydrothermally modified hardwoods. International Biodeterioration & Biodegradation, 86, 71-78.

Kiran OE, Comlekcioglu U, Dostbil N, 2006. Some microbial enzymes and their use in industry. Kahramanmaras Sutcu Imam University Journal of Science and Engineering, 91, 12-19.

Kocabas A, Gümüştaş N, Gönek S, 2017. Screening of Microorganisms Producing Xylanase from Soil and Partial Characterization of Xylanase. Karaelmas Journal of Science and Engineering, 7 (2), 503-508.

Krengel U, Dijkstra BW, 1996. Three-dimensional Structure of Endo-1, 4-β-xylanase I from *Aspergillus niger*: Molecular Basis for its Low pH Optimum. Journal of molecular biology, 2631, 70-78.

Kulkarni N, Shendye A, Rao M, 1999. Molecular and biotechnological aspects of xylanases. FEMS microbiology reviews, 234, 411-456.

Kumar CG, Joo HS, Choi JW, Koo YM, Chang CS, 2004. Purification and characterization of an extracellular polysaccharide from haloalkalophilic *Bacillus* sp. I-450. Enzyme and microbial technology, 347, 673-681.

Madigan MT, Martinko JM, Brock, TD, 2006. Brock biology of microorganisms. Upper Saddle River, NJ: Pearson Prentice Hall.

Miller GL, 1959. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. Analytical Chemistry, 313, 426-428.

Niehaus F, Bertoldo C, Kähler M, Antranikian G, 1999. Extremophiles as a source of novel enzymes for industrial application. Applied Microbiology and Biotechnology, 516, 711-729.

Roy N, Habib MR, 2009. Isolation and characterization of Xylanase producing strain of *Bacillus cereus* from soil. Iranian Journal of Microbiology, 49-53.

Srivastava RC, Madamwar DB, Vyas VV, 1987. Activation of enzymes by reversed micelles. Biotechnology and bioengineering, 297, 901-902.

Subramaniyan S, Prema P, 2002. Biotechnology of microbial xylanases: enzymology, molecular biology, and application. Critical reviews in biotechnology, 221, 33-64.

Ulucay O, 2018. Purification, production and investigation of commercial use of 1,4-β-endo xylanase in various *Bacillus* species isolated from thermal resources. Kafkas University Graduate School of Natural and Applied Sciences, Doctoral Thesis (Printed).

Ulucay O, Gormez A, Ozic C, 2021. Identification, Characterization, and Hydrolase Producing Performance of Thermophilic Bacteria: Geothermal Hot Springs in Eastern and Southeastern Anatolia Region of Turkey *researchsquare (submitted)*. doi:10.21203/rs.3.rs-348608/v1

Wainø M, Ingvorsen K, 2003. Production of beta-xylanase and beta-xylosidase by the extremely halophilic archaeon *Halorhabdus utahensis*. Extremophiles, 72, 87-93.

Zeikus JG, 1979. Thermophilic bacteria: ecology, physiology and technology. Enzyme and Microbial Technology, 14, 243-252.