Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 12(1): 133-141, 2022
Journal of the Institute of Science and Technology, 12(1): 133-141, 2022

ISSN: 2146-0574, eISSN: 2536-4618

Biyoloji / *Biology* **DOI: 10.21597/jist.948813**

Araştırma Makalesi / *Research Article*

Geliş tarihi / Received: 07.06.2021 **Kabul tarihi / Accepted: 12.11.2021**

Atıf İçin: Bayram S, Aydoğan MN, 2021. Çok Yönlü Polisakkarit Ayrıştırıcı Alkali-toleranslı veya Alkalifilik *Bacillus* Suşlarının Aranması. Journal of the Institute of Science and Technology, 12(1): 133-141.

To Cite: Bayram S, Aydoğan MN, 2021. Searching for Versatile Polysaccharide-Degrading Alkali-tolerant or Alkaliphilic *Bacillus* Strains. Journal of the Institute of Science and Technology, 12(1): 133-141.

Searching for Versatile Polysaccharide-Degrading Alkali-tolerant or Alkaliphilic *Bacillus* Strains

Sinan BAYRAM[1], Mehmet Nuri AYDOĞAN[2]

ABSTRACT: The plant-derived polysaccharides (Cellulose, xylose and amylose, etc.) are the most abundant renewable raw materials in nature. Cellulose and xylose are the predominant carbohydrate polymer components of the plant cell walls and the most abundant biopolymers in the world. Another plant-derived polysaccharide, starch is found in plant tubers, roots and seed endosperms as a major carbohydrate reserve. In this study, it was aimed to find multi-enzyme producer bacteria strains in terms of industrially important enzymes such as cellulase, xylanase and amylase. For this purpose, isolated *Bacillus* strains from different samples were qualitatively evaluated for cellulase, xylanase and amylase enzyme production potentials. The isolates that have the highest enzyme activity were selected for biochemical tests, molecular and phenotypic characterization. As a result of these characterization process, SB57, SB104, SB155, SB178, SB197 and SB199 strains were identified as *Bacillus pumilus* and SB118, SB138 strains were identified as *Bacillus safensis*. In addition to these strains, SB120 and SB147 strains were identified as *Bacillus aerius* and *Bacillus licheniformis* respectively. 16S rDNA sequence analysis results of these *Bacillus* strains were deposited in NCBI GenBank® under accession number KT371465 - KT371474 respectively.

Keywords: *Bacillus*, Alkali-tolerant, Alkaliphilic, cellulase, xylanase, amylase

[1]Sinan BAYRAM (**Orcid ID**: 0000-0002-2156-1566), Bayburt Üniversitesi, Sağlık Hizmetleri Meslek Yüksekokulu, Tıbbi Hizmetleri ve Teknikler Bölümü, Bayburt, Türkiye
[2] Mehmet Nuri AYDOĞAN (**Orcid ID**: 0000-0001-7518-4746), Atatürk Üniversitesi, Fen Fakültesi, Biyoloji Bölümü, Erzurum, Türkiye

*__Sorumlu Yazar/Corresponding Author__: Sinan BAYRAM, e-mail: sbayram@bayburt.edu.tr

*Bu makale Sinan BAYRAM'ın doktora tezinden üretilmiştir.

INTRODUCTION

Enzymes are biological catalysts that accelerate or catalyze, chemical reactions in living organisms. These biological catalysts, which can only be synthesized by living organisms, regulate reactions outside the cell as well as within the cell (Kagan and Li, 2003). The use of enzymes in daily life is nearly as old as the history of the civilization. Humans have been unwittingly benefited from the catalytic properties of enzymes for thousands of years in many processes such as the fermentation of bread, yogurt and cheese, the production of various alcoholic beverages and leather tanning (Kirk, 2002; Cherry and Fidantsef, 2003). It is reported that, the enzyme market reached annually 5.5 billion dollars in 2018, all over the world and it is estimated that this figure will reach 7 billion dollars by 2023 (Mishra et al., 2020).

Cellulose, which is one of the basic components of the cell wall in plants, is undoubtedly of great importance among the most abundant structural polysaccharides in nature. According to calculations made from various sources, plants are reported to produce 180 billion tons of cellulose on a global scale each year (Taylor, 2007) and therefore undoubtedly cellulose is one of the most abundant renewable energy sources in the world (Reese and Mandels, 1984; Coughlan, 1985).

Cellulose is formed by the linear bonding of approximately 15000 glucose units with β 1-4 glycosidic bonds. Since it can be fermented and used as a substrate in biotechnological applications, cellulose is accepted as the most abundant carbon source in the world. Enzymes involved in hydrolysis of cellulose are called "cellulase" (Li et al., 2019). It is stated that three different enzymes act synergistically in the conversion of cellulose to ß-glucose. In this process, the enzymes that use cellulose as a substrate are endoglucanases (EC 3.2.1.4), exoglucanases (EC 3.2.1.91) and ß-glucosidases (EC 3.2.1.21) (Podrepšek et al., 2019).

Xylans are the second most abundant polysaccharide in nature made from units of xylose that are found in plant cell walls and some algae. Xylanases are a class of hydrolytic enzymes which randomly cleave the linear polysaccharide *β*-1,4-xylan into xylose (Hammed et al., 2013). Xylanases are glycosidases (*O*-glycoside hydrolases, EC 3.2.1.x) which are capable of cleaving the *β*-1,4 backbone of the complex plant cell wall polysaccharide xylan (Collins et al., 2005). Because of their catalytic properties, xylanases are widely used for industrial purposes including juice clarification, bioethanol production, dough processing, textile manufacturing, paper bleaching and feed additive etc. Selected strains to produce xylanase enzyme on industrial scales are generally isolated from plant pathogens. The xylanases used in industrial production are mostly isolated from bacterial and fungal strains (Beg et al., 2001; Chen et al., 2015; Saadat, 2017). In the food industry, xylanases are used for clarifying fruit juices and reducing viscosity. In the production of wine, xylanase enzyme is also used for increasing quality and clarification of maceration liquid. The xylanase enzyme is used to increase extraction efficiency, product quality and process performance in corn oil and olive oil production (Bhat, 2000).

The main purpose of using xylanase enzyme in the production of dough and bakery products is arabinoxylan, which is found in cereals and constitutes approximately 60-70% of the grain. Arabinoxylan that is the target substrate of the xylanase is about 60-70% of the cereal flour (Aehle, 2006). The use of xylanase enzyme in the production of doughs and bakery products increases the dough strength and at the same time gives the dough elasticity properties. These processes make it easier to knead the dough and increase the quality of the dough (Bhat, 2000; Dutron et al., 2011).

In the production process of feeds of farm animals such as poultry, pigs and ruminants, the xylanase enzyme has a great importance. The xylanase enzyme is used to reduce polysaccharide content, which is found in products such as barley and wheat, which are mostly used in feed production, and

which is difficult to digest. With the use of xylanase, the digestibility of animal feeds is increased and as a result, the meat and milk yield is increased in animals (Bhat, 2000; Mathlouthi et al., 2003). In addition, in the textile industry, the xylanase enzymes are widely used in the process of pooling industrially important herbal products such as linen, ramie and hemp (Sharma, 1987; Prade, 1995; Beg et al., 2001).

As an important storage polysaccharide starch is a polymer made up of glucose units connected by glycosidic bonds and is generally found in seeds, roots and tubers of large plants. There are two different types of glucose polymers in the starch: amylose and amylopectin. In amylose, which makes up approximately 30% of starch, α 1-4 glycosidic bonds are seen, while amylopectin, which makes up about 70%, has α1-6 glycosidic bonds (Barsby et al., 2001). Starch has a very complex structure and two different enzyme groups take part in the separation of starch into oligosaccharides and smaller building blocks. Endohydrolases (Endo-acting) and exohydrolases (Exo-acting) (Polaina and MacCabe, 2007). Among the commercially used enzymes, amylases have an important place. Amylase enzyme is used in many industrial fields such as food, textile, pharmaceutical, paper and detergent industries. The first use of this enzyme as a pharmaceutical supplement was in 1984 for the treatment of digestive disorders (Gupta et al., 2003; Debarati et al., 2014).

Alkaliphiles are a group of extremophilic microorganisms that grow optimally in alkaline environments at pH values above 9.0. Alkaline enzymes produced by alkaliphilic microorganisms are used in many fields such as detergent industry, tanning of the leather, food industry, cosmetics and pharmacology (Horikoshi, 1999).

In this study, alkaliphyl and alkalitolerant *Bacillus* strains has been isolated from different origins and these strains has been investigated for cellulose, xylanase and amylase enzyme production potentials. The strains with the highest enzyme activity were selected for physiological, biochemical and morphological and molecular characterization procedures. Based on the information obtained from these results, isolates were registered in the NCBI database.

MATERIALS AND METHODS

Isolation of Bacterial Strains

The microorganisms used in this study were isolated from different sources. For this purpose, soil samples were taken from the rhizosphere and the surface, in addition, rotten fruit samples, decaying plants and paper samples were used for purification of microorganisms. The samples taken from nature were transferred to sterilized 50 mL falcon tubes and brought to the laboratory. Samples diluted by serial dilution using physiological saline in the laboratory were inoculated into appropriate media (Ghadiri et al., 2021).

Qualitative determination of cellulase enzyme activity

PYC (Peptone, yeast and carboxy-methyl cellulose) medium (pH: 9.0) was used to determine the cellulase enzyme activity of isolated *Bacillus* strains (Figure 1a). For this purpose, microorganisms were inoculated in a straight line on the prepared solid PYC medium which was composed of 10.0 g L^{-1} carboxy-methyl cellulose (CMC), 5.0 g L^{-1} peptone, 5.0 g L^{-1} yeast extract, 1.0 g L^{-1} KH_2PO_4, 0.2 g L^{-1} $MgSO_4.7H_2O$, 15.0 g L^{-1} agar-agar. All isolates were incubated for 72 h at 30 °C. At the end of the incubation period, 3 mL, 0.1% Congo red was added to the media and waited for 20 minutes and staining was performed in this way. After the staining process, 5mL, 1M NaCl was added in order to remove the dye from the medium and waited for 15 minutes. Yellow zones were observed around microorganisms capable of degrading cellulose and these bacteria were recorded as cellulase positive (Kim et al., 2005; Nelson et al., 2021).

Qualitative determination of xylanase enzyme activity

All isolates were incubated in xylan medium (pH: 9.0) was composed of 5.0 g L^{-1} oat spelt xylan, 5.0 g L^{-1} peptone, 1.0 g L^{-1} yeast extract, 1.0 g L^{-1} KH_2PO_4, 0.2 g L^{-1} $MgSO_4.7H_2O$, 0.1 g L^{-1} $CaCl_2.2H_2O$, 1.0 g L^{-1} K_2HPO_4, 15.0 g L^{-1} agar-agar. After these *Bacillus* strains were inoculated in a straight line on the prepared solid media, they were left to incubate at 30 ° C for 72 hours. Then, plates were flooded with 0.1% Congo red (Sigma-Aldrich) for 15 min. Finally, 10 mL, 1 M NaCl was added to the medium and waited for 15 minutes to de-staining (Figure 1b). Strains showing discoloration around the samples were selected as xylanase positive bacteria (Teather and Wood, 1982; Bozoglu et al., 2015).

Qualitative determination of amylase enzyme activity

To determine the amylase enzyme activity of isolated *Bacillus* strains, M9 Starch agar (pH: 9.0) was used (Figure 1c). All isolates were incubated in M9 Starch agar (pH: 9.0) was composed of 10.0 g L^{-1} soluble starch, 3.0 g L^{-1} peptone, 6.0 g L^{-1} $Na_2HPO_4.7H_2O$, 3.0 K_2HPO_4, 1.0 g L^{-1} NH_4Cl, 0.5 g L^{-1} NaCl, 0.24 g L^{-1} $MgSO_4.7H_2O$, 0.24 g L^{-1} $CaCl_2.2H_2O$, 15.0 g L^{-1} agar-agar). After the microorganisms were inoculated in a straight line on the prepared solid media, they were left to incubate at 30 ° C for 72 hours. At the end of the incubation period, the media are stained with iodine vapor. After this process, while the starch-containing medium was dyed dark blue, colorless zones were observed around the microorganisms capable of degrading starch and these bacteria were recorded as amylase positive (Hols et al., 1994; Al-Dhabi et al., 2020).

Physiological, Biochemical and Morphological Characterization of *Bacillus* strains

In order to carry out the physiological, biochemical and morphological characterization of the enzyme producer *Bacillus* strains, features such as endospore formation, colony color, catalase activity, gram stain and motility etc. were determined (Table 1 and figure 1d) (Harley and Prescott, 2002; Whitman et al., 2012).

Isolation of Total Genomic DNA and PCR amplification of 16S rDNA

Total genomic DNA extraction procedures was modified from Wilson (2001). The isolated DNA pellets were dissolved 100 mL of TE buffer and stored at -20 °C until being used (Figure 2a). Before the amplification process, in order to perform the reaction, a 50 µl master mix was prepared for each sample. For this purpose, 2 µL of extracted genomic DNA was added to 48 µL of mastermix for the PCR reaction mixture (Figure 2b). Universal primers 27F and 1492R were used in this amplification process. PCR amplification process of 16S rRNA was carried out with small modification described by Bayram (2021). Then, the 16S rRNA nucleotide sequence analysis results of these *Bacillus* strains were determined (Macrogen, Korea). The nucleotide sequences of the 16S rRNA amplicons were subjected to BLAST analysis with the NCBI GenBank® database. The sequence analysis results of these *Bacillus* strains have been deposited in GenBank® Data Library (Table 1).

RESULTS AND DISCUSSION

Bacillus are endospore-forming, gram-positive, rod shape aerobic bacteria and this genus are known as a one of the most important industrial enzyme sources (Gordon et al., 1973; Turnbull et al., 1991; Nicholson, 2002; Merghni et al., 2014). In this study, it has been investigated xylanase cellulase and amylase enzyme activities of alkalophil and alkaline-tolerant *Bacillus* strains isolated from different origins (Figure 1). Among the isolated strains, 10 strains were observed to have enzyme activity in terms of cellulase, xylanase and amylase enzymes, and these strains were selected for 16S rDNA sequence analysis. Sequence analysis results are given in table 1.

It is seen that there are numerous studies in the literature about industrial enzyme production with the use of microorganisms (Adrio and Demain, 2014; Singh et al., 2016; Naureen, 2021). In a study conducted by Ariffin et al., (2014) the cellulase enzyme production potential of the local isolate *Bacillus pumilus* EB3 was evaluated (Ariffin et al., 2014). In that study, after determining the enzyme activity qualitatively, the cellulase enzyme was purified and characterized by anion exchange chromatography. It has been stated by the researchers that the purified carboxymethyl cellulase enzyme had a molecular weight range of 30-65 kDa and the optimum temperature is 60 °C and the optimum pH value is 6.0.

In a study conducted by Thite et al., (2020) different agrowaste biomasses such as wheat bran and citrus peel were used as fermentation substrates. In this study, the xylanolytic and pectinolytic enzyme activities of *Bacillus safensis* M35 and *Bacillus altitudinis* J208 strains were evaluated by the Central Composite Design of Response Surface Method. As a result of these assays, the content of optimized production medium (OPM) was determined as 1.57% wheat bran and 1.26% citrus peel. Xylanolytic and pectinolytic enzyme activities for *Bacillus safensis* M35 strain were determined as 15.10 U and 411.58 U, respectively.

Table 1. Physiological, biochemical and morphological and molecular characterization results of *Bacillus* strains

No	Strain	Species	Morphology	Gram Stain	Catalase	Endospore formation		Accession Number	Enyzme Activiy
						Formation	Location		
1	SB57	*B. pumilus*	Rod-shaped	Gram +	Catalase +	+	Subterminal	KT371465	Xylanase
2	SB104	*B. pumilus*	Rod-shaped	Gram +	Catalase +	+	Subterminal	KT371466	Cellulase
3	SB118	*B. safensis*	Rod-shaped	Gram +	Catalase +	+	Terminal	KT371467	Xylanase
4	SB120	*B. aerius*	Rod-shaped	Gram +	Catalase +	+	Terminal	KT371468	Cellulase
5	SB138	*B. safensis*	Rod-shaped	Gram +	Catalase +	+	Subterminal	KT371469	Cellulase
6	SB147	*B. licheniformis*	Rod-shaped	Gram +	Catalase +	+	Teminal	KT371470	Cellulase
7	SB155	*B. pumilus*	Rod-shaped	Gram +	Catalase +	+	Subterminal	KT371471	Amylase
8	SB178	*B. pumilus*	Rod-shaped	Gram +	Catalase +	+	Subterminal	KT371472	Amylase
9	SB197	*B. pumilus*	Rod-shaped	Gram +	Catalase +	+	Subterminal	KT371473	Cellulase
10	SB199	*B. pumilus*	Rod-shaped	Gram +	Catalase +	+	Subterminal	KT371474	Xylanase

In a study performed by Nelson et al., (2021), the cellulolytic capacities of *Bacillus* strains isolated from the intestinal flora of *Schistocerca gregaria* were investigated. The cellulase enzyme activities of these isolated bacteria were determined by zone clearance assay, and the Index Cellulolytic Activity values (ICA values) of *Bacillus safensis* strain MED1 and *Bacillus safensis* strain CACO strains were determined as 1.146 ± 0.109 and 0.8442 ± 0.09203, respectively.

In another study conducted by Yoon et al., (2014), it was aimed to isolation and identification of xylanolytic bacteria from *Ceratotherium simum* feces. It has been stated that *Bacillus pumilus* H10-1 strain had the highest xylanase activity among isolated 67 strains and the optimum conditions are pH 9 at 30°C and the incubation period is 7 days (Yoon et al., 2014).

In addition, in a study conducted by Ayansina et al., (2017) amylase enzyme activities of microorganisms isolated from Cassava shells were determined and the amylase enzyme partially purified from these isolated microorganisms was characterized. In that study, compared to other microorganisms, *Bacillus pumilus* was found to have the highest amylase enzyme activity (44.6 units mL^{-1} on day 3).

In addition to these studies, cellulase enzyme activities of thermophilic bacteria isolated from West Coast hot spring in Rat-nagiri District of Maharashtra (India) were determined by Shajahan et al., (2017). In that study, optimal conditions were determined for *Bacillus licheniformis* NCIM5556 strain using the response surface methodology (RSM) and it was reported that the highest cellulase enzyme production in a 7 L volume fermentor was determined as 42.99 IU mL^{-1} (Shajahan et al., 2017).

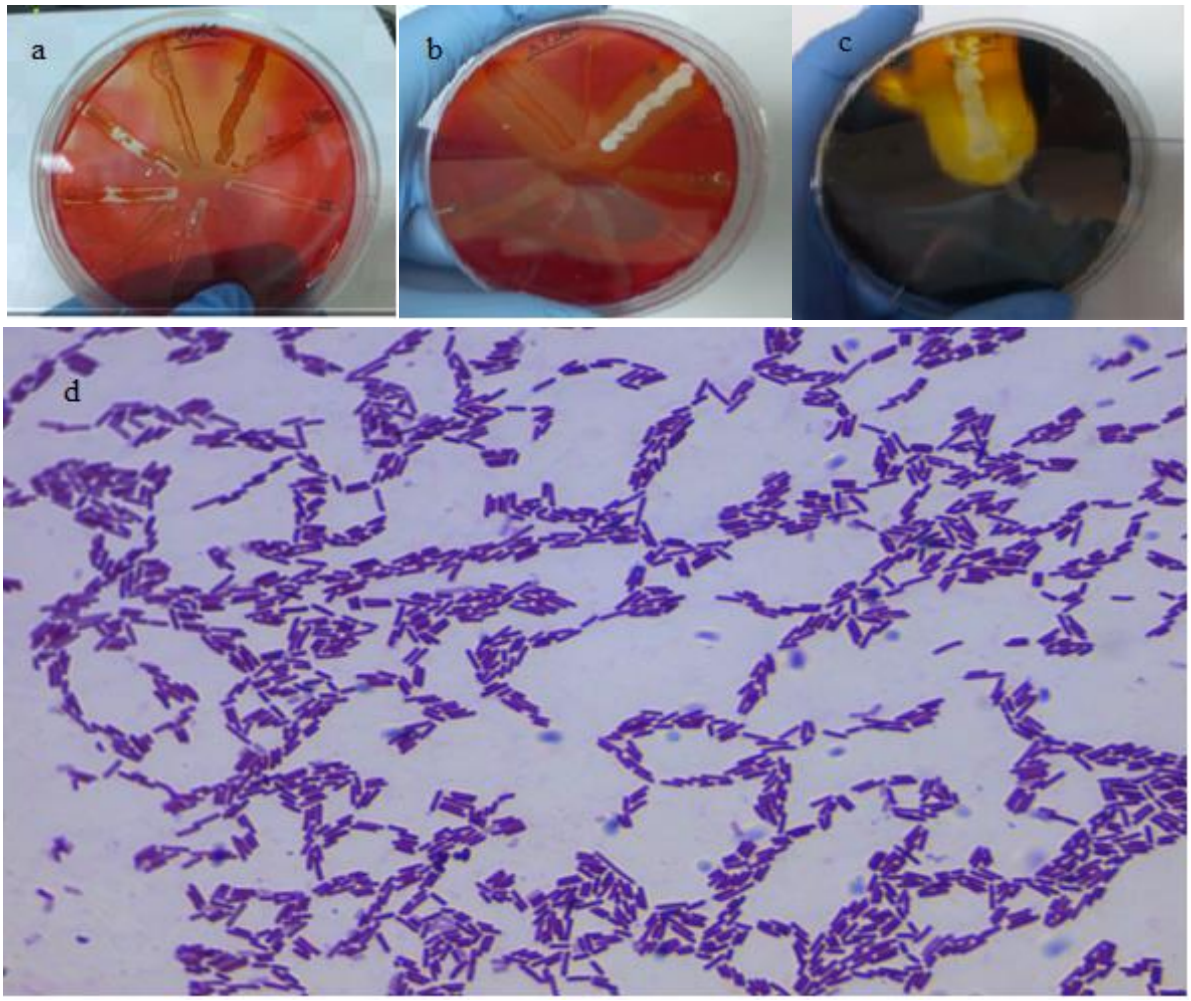
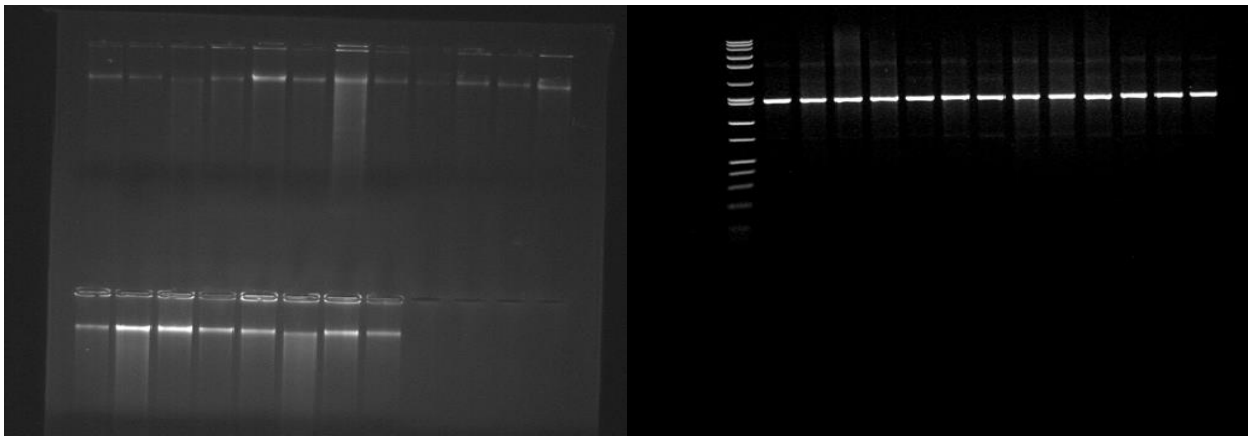


Figure 1. Qualitative determination of bacterial cellulase (a), xylanase (b) amylase (c) enzyme activities and immersion image of a *Bacillus* strain (d)



(a) (b)

Figure 2. Agarose gel electrophoresis image of genomic DNA (a) and agarose gel electrophoresis image of 16S rDNA (b)

CONCLUSION

In this presented study, isolated alkaliphil and alkalitolerant *Bacillus* strains from various sources such as soil and decaying vegetation have been evaluated for cellulase, xylanase and amylase enzyme activity. In this way, industrial enzyme production potentials (cellulase, xylanase and amylase) of these *Bacillus* strains were qualitatively evaluated. During these processess, it was aimed to find versatile polysaccharide degrading alkali-tolerant or alkaliphilic *Bacillus* strains. Enzyme activities were evaluated qualitatively and the bacterial strains observed in the largest zone were selected for 16S rDNA sequence analysis. Among these strains, 6 *Bacillus pumilus*, 2 *Bacillus safensis*, 1 *Bacillus licheniformis* and 1 *Bacillus aerius* were identified. The obtained results were evaluated and among these isolated strains, no strain was found showing high activity in terms of two or more enzymes. Finally, sequence analysis results of these strains have been deposited in NCBI GenBank® Data Library under accession number KT371465 - KT371474 respectively. Following this study, it is planned to partial purification of these enzymes, determining the molecular weights and determine the stability against parameters such as pH and temperature in future studies.

ACKNOWLEDGEMENTS

This study is a part of PhD dissertation written by Sinan BAYRAM (Doctoral Programme of Atatürk University, Science Institute, Erzurum).

Conflict of Interest

The authors declare that there is no conflict of interest.

Author's Contributions

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

REFERENCES

Adrio JL, Demain AL, 2014. Microbial enzymes: tools for biotechnological processes. Biomolecules, 4(1): 117-139.

Aehle W, 2007. Enzymes in industry: production and applications. John Wiley & Sons. Hoboken, NJ, USA.

Al-Dhabi NA, Esmail GA, Ghilan AKM, Arasu MV, Duraipandiyan V, Ponmurugan K, 2020. Isolation and purification of starch hydrolysing amylase from *Streptomyces* sp. Al-Dhabi-46 obtained from the Jazan region of Saudi Arabia with industrial applications. Journal of King Saud University-Science, 32(1): 1226-1232.

Ariffin H, Abdullah N, Umi-Kalsom MS, Shirai Y, Hassan, MA, 2006. Production and characterization of cellulase by *Bacillus pumilus* EB3. International Journal of Engineering Technologies, *3*(1): 47-53.

Ayansina ADV, Adelaja AO, Mohammed SSD, 2017. Characterization of amylase from some *Aspergillu*s and *Bacillus* species associated with cassava waste peels. Advances in Microbiology, *7*(04): 80.

Barsby TL, Donald AM, Frazier PJ, 2001. Starch: Advances in structure and function. Royal Society of Chemistry. Cambridge. UK.

Bayram S, 2021. Production, purification, and characterization of *Streptomyces* sp. strain MPPS2 extracellular pyomelanin pigment. Archives of Microbiology, 203: 4419–4426 https://doi.org/10.1007/s00203-021-02437-w

Beg Q, Kapoor M, Mahajan L, Hoondal GS, 2001. Microbial xylanases and their industrial applications: a review. Applied microbiology and biotechnology, 56(3-4): 326-338.

Bhat MK, 2000. Cellulases and related enzymes in biotechnology. Biotechnology advances, 18(5): 355-383.

Bozoglu C, Hundur S, Alaylar B, Karadayi M, Gulluce M, 2015. Isolation and Molecular Characterization of Thermophilic Bacteria with Xylanase Activity from Thermal Springs in Erzurum. Journal of Life Sciences and Technologies, 3(1): 32-36

Chen CC, Ko TP, Huang JW, Guo RT, 2015. Heat-and Alkaline-Stable Xylanases: Application, Protein Structure and Engineering. ChemBioEng Reviews, 2(2): 95-106.

Cherry JR, Fidantsef AL, 2003. Directed evolution of industrial enzymes: an update. Current opinion in biotechnology, 14(4): 438-443.

Collins T, Gerday C, Feller G, 2005. Xylanases, xylanase families and extremophilic xylanases. FEMS microbiology reviews, 29(1): 3-23.

Coughlan MP, 1985. Cellulose hydrolysis: the potential, the problems and relevant research at Galway. Biochemical Society Transactions, 13 (2): 405–406. https://doi.org/10.1042/bst0130405

Debarati H, Paswan KA, Abhik C, Ethina B, Malini B, 2014. Characterization and cell immobilization of a potent amylase producing mesophilic soil bacteria *Bacillus cereus* strain BRSC-S-A26MB. Journal of Mycopathological Research, *52*(1): 11-19.

Dutron A, Georis J, Genot B, Dauvrin T, Collins T, Hoyoux A, Feller G, 2012. Use of family 8 enzymes with xylanolytic activity in baking. Granted Patents US8192772 (2012), EP1549147B1 (2011), CN1681392B (2010), DE60336153 D1 (2011), CA 2498014C (2011), ES2360942 (2011), DE60336153D1 (2011)Gordon RE, Haynes WC, Pang CHN, 1973. The genus *Bacillus* (No. 427). Agricultural Research Service, US Department of Agriculture.

Ghadiri E, Naghavi NS, Ghaedi K, 2021. Gene Production and Characterization of *Bacillus Subtilis* Cellulase Collected from Central-Northern Iran Forests. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 91: 543–548.

Gupta R, Gigras P, Mohapatra H, Goswami VK, Chauhan B, 2003. Microbial α-amylases: a biotechnological perspective. Process biochemistry, 38(11): 1599-1616.

Hammed AM, Jaswir I, Amid A, Alam Z, Asiyanbi-H, TT, Ramli N, 2013. Enzymatic hydrolysis of plants and algae for extraction of bioactive compounds. Food Reviews International, 29(4): 352-370.

Harley JP, Prescott LM, 2002. Laboratory Exercises in Microbiology, 5th ed. New York: McGraw-Hill Press, p466.

Hols P, Ferain T, Garmyn D, Bernard N, Delcour J, 1994. Use of homologous expression-secretion signals and vector-free stable chromosomal integration in engineering of *Lactobacillus plantarum* for alpha-amylase and levanase expression. Applied and Environmental Microbiology, *60*(5): 1401-1413.

Horikoshi K, 1999. Alkaliphiles: some applications of their products for biotechnology. Microbiology and molecular biology reviews, 63(4): 735-750.

Kagan HM, Li W, 2003. Lysyl oxidase: properties, specificity, and biological roles inside and outside of the cell. Journal of cellular biochemistry, *88*(4):660-672.

Kim JY, Hur SH, Hong JH, 2005. Purification and characterization of an alkaline cellulase from a newly isolated alkalophilic *Bacillus sp.* HSH-810. Biotechnology letters, *27*(5): 313-316.

Kirk O, Borchert TV, Fuglsang CC, 2002. Industrial enzyme applications. Current opinion in biotechnology, 13(4): 345-351.

Li X, Xu Q, Shen H, Guo Y, Wu M, Peng Y, Xie H, 2019. Capturing CO_2 to reversible ionic liquids for dissolution pretreatment of cellulose towards enhanced enzymatic hydrolysis. Carbohydrate polymers, *204*: 50-58.

Mathlouthi N, Juin H, Larbier M, 2003. Effect of xylanase and β-glucanase supplementation of wheat-or wheat-and barley-based diets on the performance of male turkeys. British poultry science, 44(2): 291-298.

Merghni A, Leban N, Behi A, Bakhrouf A, 2014. Evaluation of the probiotic properties of *Bacillus* spp. strains isolated from Tunisian hypersaline environments. African journal of microbiology research, *8*(4): 398-405.

Mishra P, Mishra J, Dwivedi SK, Arora NK, 2020. Microbial Enzymes in Biocontrol of Phytopathogens. In Microbial Enzymes: Roles and Applications in Industries (pp. 259-285). Springer, Singapore.

Naureen I, 2021. A Review on Microbial Enzymes, Synthesis, Biological Role, Current Applications and Future Perspectives. Scholars Bulletin, *7*(3): 44-48.

Nelson K, Muge E, Wamalwa B, 2021. Cellulolytic *Bacillus* species isolated from the gut of the desert locust *Schistocerca gregaria*. Scientific African, *11*, e00665. https://doi.org/10.1016/j.sciaf.2020.e00665

Nicholson WL, 2002. Roles of *Bacillus* endospores in the environment. Cellular and Molecular Life Sciences, 59(3): 410-416.

Podrepšek GH, Knez Ž, Leitgeb M, 2019. Activation of cellulase cross-linked enzyme aggregates (CLEAs) in scCO2. The Journal of Supercritical Fluids, *154*: 104629.

Polaina J, MacCabe AP, 2007. Industrial enzymes. (pp. 531-547). Dortrecht: Springer.

Prade RA, 1995. Xylanases: from biology to biotechnology. Biotechnology and Genetic Engineering Reviews, 13: 100–131.

Reese ET, Mandels M, 1984. Rolling with the times: production and applications of *Trichoderma reesei* cellulase. In Annual reports on fermentation processes. 7: 1-20

Saadat F, 2017. A review on chimeric xylanases: methods and conditions. 3 Biotech, 7(1): 67.

Shajahan S, Moorthy IG, Sivakumar N, Selvakumar G, 2017. Statistical modeling and optimization of cellulase production by *Bacillus licheniformis* NCIM 5556 isolated from the hot spring, Maharashtra, India. Journal of King Saud University-Science, *29*(3): 302-310.

Sharma HSS, 1987. Enzymatic degradation of residual noncellulosic polysaccharides present on dew-retted flax fibers. Applied Microbiology and Biotechnology, 26: 2714–2723.

Singh R, Kumar M, Mittal A, Mehta PK, 2016. Microbial enzymes: industrial progress in 21st century. 3 Biotech, *6*(2): 1-15. https://doi.org/10.1007/s13205-016-0485-8

Taylor NG, 2007. Identification of cellulose synthase AtCesA7 (IRX3) in vivo phosphorylation sites—a potential role in regulating protein degradation. Plant molecular biology, *64*(1): 161-171.

Teather RM, Wood PJ, 1982. Use of Congo red-polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. Applied and environmental microbiology, 43(4): 777-780.

Thite VS, Nerurkar AS, Baxi NN, 2020. Optimization of concurrent production of xylanolytic and pectinolytic enzymes by *Bacillus safensis* M35 and *Bacillus altitudinis* J208 using agro-industrial biomass through Response Surface Methodology. Scientific reports, *10*(1): 1-12.

Turnbull PC, Kramer JM, Melling J, 1991. *Bacillus*. Manual of clinical microbiology, 5: 296-303.

Whitman WB, Goodfellow M, Kämpfer P, Busse HJ, Trujillo ME, Ludwig W, Suzuki KI, 2012. Bergey's manual of systematic bacteriology: The Actinobacteria. Springer. New York. USA

Wilson, K. (2001). Preparation of genomic DNA from bacteria. Current protocols in molecular biology, 56(1): 2-4.

Yoon YM, An GH, Kim JK, Ahn SH, Cha YL, Yang J, Choi IH. 2014. Xylanase activity of *Bacillus pumilus* H10-1 isolated from *Ceratotherium simum* feces. Korean Society for Biotechnology and Bioengineering Journal, *29*(5): 316-322.