Cilt 11, Sayı 4, Sayfa 30-35, 2022 Volume 11, Issue 4, Page 30-35, 2022

Araştırma Makalesi https://doi.org/10.46810/tdfd.1169601 Research Article

Türk Doğa ve Fen Dergisi
Turkish Journal of Nature and Science
www.dergipark.gov.tr/tdfd

Isolation of a Novel Amylase Producing *Brachybacterium paraconglomeratum* Strain FAD4 and Optimization of the Enzyme Production Conditions

Dilsat Nigar COLAK[1*]

[1]Giresun University, Dereli Vocational School, Department of Forestry, Giresun, 28950 Türkiye
Dilsat Nigar COLAK ORCID No: 0000-0001-9544-3733

**Corresponding author: dilsat.nigar@giresun.edu.tr*

(Received: 01.09.2022, Accepted: 17.10.2022, Online Publication: 28.12.2022)

Keywords
Amylase, Brachy-bacterium, Starch

Abstract: A novel amylase producing bacterium FAD4 was isolated from the wastewater of a textile factory located in Soke (Aydın/Turkey). The amylase production ability of gram positive, coccoidal FAD4 strain was confirmed with plate assay. Morphological and 16S rRNA sequence analyses revealed that FAD4 belongs to the genus *Brachybacterium* with a sequence similarity of 99.8% with *B. paraconglomeratum.* The optimal conditions for amylase production were determined as 72 h at 30 °C with supplementation of 1% starch. Optimum temperature and pH of the amylase were 50 °C and 7.0 respectively. Different starch, carbon and nitrogen sources were investigated for amylase production. A high enzyme production was observed with 1% potato starch and among nitrogen sources peptone was induced the production of amylase. Lactose, galactose, and fructose were also increased the enzyme production as carbon sources.

Yeni Bir Amilaz Üreticisi *Brachybacterium paraconglomeratum* FAD4'ün İzolasyonu ve Enzim Üretim Koşullarının Optimizasyonu

Anahtar Kelimeler
Amilaz, Brachy-bacterium, Nişasta

Öz: Yapılan bu çalışmada Aydın (Türkiye)'ın Söke ilçesinde bulunan bir tekstil fabrikasının atık suyundan amilaz üreten bir suş; FAD4 izole edilmiştir. Gram pozitif ve kokoid olan FAD4'ün amilaz üretme yeteneği petri deneyi ile belirlenmiştir. FAD4'ün morfolojik özellikleri ve 16S rRNA dizin analizi sonuçları bu suşun %99.8 oranında *Brachybacterium paraconglomeratum* türüne benzediğini göstermiştir. Amilaz üretimi için optimum koşullar %1 nişasta ilavesi ile 30 °C'de 72 saat olarak belirlenmiştir. FAD4 amilazının optimum aktivite sıcaklığı 50 °C ve pH'sı 7.0'dir. Amilaz üretimi için farklı nişasta, karbon ve azot kaynaklarının etkisi incelenmiştir. %1 patates nişastası varlığında yüksek oranda amilaz üretimi gözlenmiştir. Ayrıca azot kaynaklarından peptonun amilaz üretimini artırdığı, karbon kaynaklarından ise laktoz, galaktoz ve fruktozun enzim üretimini artırdığı gözlenmiştir.

1. INTRODUCTION

Microbial enzymes have been used in industrial applications for decades because of their high activity and stability. They also cost-effective and economically friendly. Many of these microbial enzymes are used in various industries such as food, detergent, textile, paper, medicine etc. [1]. Amylases (EC 3.2.1.1) are one of the most significant enzymes used in industry. These extracellular enzymes catalyse the breakdown of starch into glucose and oligosaccharides via hydrolysing the internal α-1,4 glycosidic linkages in a random manner [2]. Sequence similarity analyses of the catalitic domain revealed that most of the α-amylases are belong to glycoside hydrolase (GH) family 13 [3]. The conserved structure of GH13 include a $(\beta/\alpha)_8$ barrel that contains the active site and extra domains can vary based on the type of amylase [4]. Although amylases can be produced by a variety of organsisms (microorganisms, animals, and plants), microorganisms are the best source for industrial applications due to mild growth conditions and cost-effectiveness [5]. However, the demand of growth conditions differ significantly depending on the producers like temperature, time, nutritional source, metal ions etc.

Starch is produced by most of the plants for energy storage and constitutes an important part of the human and animal diet. The enzymatic hydrolysis of starch has a broad range of applications in industry. α-amylases are one of the major enzymes used in starch processing and

they utilized in glucose syrup, brewing, baking as well as detergent, paper and pulp, textile, leather, and distilling industries for decades [6]. A variety of bacteria have been reported for α-amylase production. Bacillus species are the most common bacterial sources, however amylases from Geobacillus, Lactobacillus, Pseudomonas, Corynebacterium, Nesterenkonia species were also reported [5, 7, 8, 9].

In this work an amylase producing bacterium *Brachybacterium paraconglomeratum* was isolated from wastewater samples. Species of *Brachybacterium* are rod-cocci shaped, Gram-positive, nonmotile, aerobic or weakly anaerobic bacteria. They were isolated from various environments, such as garden soil, oil-contaminated sand, seawater, poultry deep litter, mouse liver, fermented cheese and seafood, and roots [10, 11, 12, 13]. There is only one report about amylase producing *Brachybacterium* that is a maltooligosaccharide-forming amylase from *Brachybacterium* sp. strain LB25 [14]. In present study we aimed to determine the optimum conditions of α-amylase production from the newly isolated strain *Brachybacterium paraconglomeratum.*

2. MATERIAL AND METHOD

2.1. Bacterial Strain

In a previous work wastewater and contaminated soil samples were collected from a textile factory located in Soke (Aydın/Turkey). Samples were diluted, inoculated into nutrient agar medium (NA, 105450, Merck Millipore) and incubated at 30 °C for 18 h. Growing colonies were subcultured into NA plates to obtain single colonies. Based on coloni morphologies 13 isolates were selected. Gram staining was performed and colony morphologies were determined using an Olympus CX21 light microscope. Amylase production capacities of the isolates were screened via plate assay. Four of the isolates (FAD2, FAD3, FAD4, and FAD12) were amylase positive. The 16S rRNA sequences of the strains were amplified by PCR using universal UNI16S-L as forward and UNI16S-R as reverse primers for identification. Sequence similarities were analysed with online databases NCBI Genbank and EzTaxon. After identification of the strains, FAD4 was choosen for amylase production.

2.2 Qualitative Screening of Amylase Production

The method of Atlas et al. [15] was used for amylase activity screening. Bacterial strains were inoculated into NA medium containing 1% (w v^{-1}) of soluble starch and incubated at 30 °C for two days. After incubation plates were flooded with 1% of Iodine solution and kept at room temperature for 5 minutes. The formation of dark-blue color represented the precence of starch and clear zones indicated the hydrolysis of starch around colonies.

2.3. Enzyme Production and Amylase Assay

Enzyme production was carried out in a shake flask containing NB medium enriched with 2% of soluble starch. 2 ml overnight culture of FAD4 was inoculated into enriched medium and incubated at 30 °C for two days. Samples were centrifuged at 11.000 rpm for 6 min and supernatant was used as crude enzyme extract.
DNS (3.5-dinitro salicyclic acid) method [16] was used for determination of amylase activity, soluble starch (0.5% w v^{-1}) in 0.05 M Tris-HCl buffer (pH:7.0) was the substrate. The reaction mixture containing 50 µl of crude enzyme and 50 µl of substrate was incubated at 50 °C for 30 min. 500 µl of DNS (1%) was added to stop the reaction and boiled in a water bath for 10 min. The release of reducing sugar was measured spectrophotometrically at 540 nm, the amount of liberated maltose was calculated by a standard curve.

2.4. Effects of Temperature and Incubaion Time on Enzyme Production

The strain FAD4 is a mesophilic bacterium, thats why it can not grow at high temperatures. Incubations at room temperature, 30 °C, and 37 °C were performed for determining optimum enzyme producing temperature. The effect of incubation time was determined by cultivating FAD4 at different times ranging from 18 hours to 4 days.

2.5. Optimum Temperature and pH of Amylase

Optimum temperature of FAD4 amylase was determined by incubating the reaction mixture including crude enzyme extract and substrate (soluble starch; 0.5% w v^{-1} in Tris-HCl buffer) at different temperatures ranging from 30 – 80 °C. The effect of pH on enzyme activity was measured by using following buffers: sodium acetate buffer (pH 5.0), sodium phosphate buffer (pH 6.0 – 8.0), Tris-HCl buffer (pH 7.0 – 9.0), glycine buffer (pH 9.0 – 12.0). Soluble starch (0.5%) was dissolved in each buffer (50 mM), mixed with enzyme and the reaction performed as mentioned above. Each experiment was carried out three times.

2.6. Effects of Starch Sources

Optimal strarch concentration was determined by supplementing NB medium with 0.5, 1, 2, and 5% (w v^{-1}) of soluble starch. The effect of different starch sources on bacterial growth and enzyme production was analysed by using 2% (w v^{-1}) of each starch source (soluble starch, corn starch, wheat starch, and potato starch) dissolved in NB medium. After inoculation with 2 ml of overnight FAD4 bacterial culture and incubation at 30 °C for 2 days, crude enzyme extract was obtained as mentioned above. Enzyme production was measured with Bradford [17] method and DNS method was used for enzyme activity.

2.7. Effects of Various Carbon and Nitrogen Sources on Enzyme Production

Various carbon sources (glucose, galactose, glycerol, sucrose, fructose, lactose, maltose, and starch) and nitrogen sources (peptone, tryptone, yeast extract, urea, casein, ammonium chloride, ammonium sulphate) were tested for enzyme production. Each source was dissolved in NB medium at a concentration of 1% w v^{-1}, and autoclave sterilized. After an incubation period of 2 days at 30 °C, crude enzyme extracts were obtained via centrifugation and enzyme activity assay was performed.

3. RESULTS AND DISCUSSION

3.1. Identification of Bacteria

Based on qualitative screening of amylase, strain FAD4 was choosen for this study because of better starch hydrolising capacity than other three amylase producing strains. FAD4 was Gram positive and the cells were round-shaped. 16S rRNA sequence analyses revealed that FAD4 has a 99.8% sequence similarity with *Brachybacterium paraconglomeratum*. Clear zones around colonies in amylase plate assay represented that FAD4 has the ability of amylase production (Figure 1).

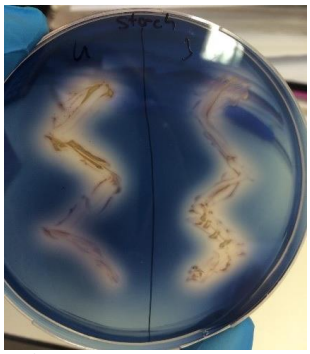

Figure 1. After washing the plates with 1% of iodine solution, formation of clear zones around strain FAD4 colonies indicates the starch hydrolysis

Microbial amylases have been used in industry for decades due to their starch hydrolysing capacity. Detergent, textile, paper, food and alcohol industries are some of the industries that amylases widely used [18]. Though most commonly used bacterial amylase sources are Bacillus species such as *B. subtilis, B. licheniformis, B. amyloliquifaciens, B. stearotherpophilus* [19], some other strains are also capable of amylase production. For example *Chromohalobacter* sp., *Caldimonas taiwanensis, Halobacillus* sp., *Haloarcula hispânica, Halomonas meridiana, Rhodothermus marinus, Geobacillus thermoleovorans, Lactobacillus fermentum* are also reported as amylase producing strains [20]. In our study we identified a new amylase producing bacterium *Brachybacterium paraconglomeratum* strain FAD4 from wastewater of a textile factory. In addition to 16S rRNA sequence similarities, the Gram staining feature and the coccoidal form is also similar with *B. paraconglomeratum*. There is only one report about amylase producing Brachybacterium presented by Doukyu et all. [14, 21]. They cloned a maltooligosaccharide-forming amylase gene from *Brachybacterium* sp. strain LB25 into *E. coli* and expressed but the specific activity of the clonned enzyme was lower than the crude enzyme extract.

3.2. Effects of Temperature and Incubaion Time on Enzyme Production

Industrial enzyme production is affected by various factors like fermentation procedure, growing media, carbon and nitrogen sources, temperature, pH, etc. *Brachybacterium paraconglomeratum* FAD4 was incubated at room temperature, 30 and 37 °C from 18 hours to 4 days and followed by crude enzyme extraction. Spectrophotometric measurements at 540 nm for protein concetration and 489 nm for amylase activity revealed that maximum yield of amylase was obtained from *B. paraconglomeratum* FAD4 at 30 °C for 72 h. When incubation period decreased to 48 hours there was only a 10% decrease observed in enzyme production, and halved when incubated for 24 h. After third day there was no increase in enzyme production. The optimum growing duration of industrial amylase producing *Bacillus* strains is range from 24 to 72 h [22]. The temperature needed for bacterial amylase production is between ~25 °C to around 100 °C [23]. Based on these data it can be said that the growing conditions of strain FAD4 is suitable for industrial applications.

3.3. Optimum Temperature and pH of Amylase

The activity and stability of the bacterial enzymes are highly related to the pH and temperature. The amylase activity of FAD4 was examined at different temperatures ranging from 30 – 80 °C, and different pH values ranging from pH 5.0 – 12.0. The optimum temperature and pH of the crude amylase extract were determined as 50 °C and pH 7.0 respectively (Figure 2). Optimum pH of the bacterial amylases were range from 4.0 (*Bacillus sp.* KR-8104) to 10.0 (*Bacillus subtilis* DM-03), and mostly 7.0. The temperature optima is varied from 33 °C (*Bacillus amyloliquefaciens*) to 135 °C (*Bacillus subtilis*) [20]. The results obtained from strain FAD4 were similar with literature.

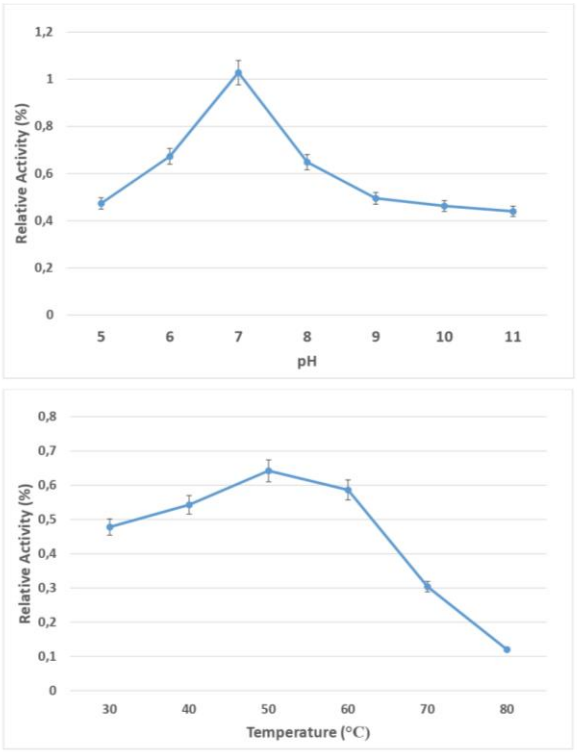

Figure 2. Optimum pH and temperature of the amylase of strain FAD4

3.4. Effects of Starch Sources on Enzyme Production

Starch concentration optimization was performed by using 0.5, 1, 2, and 5% (w v^{-1}) of soluble starch. FAD4 can grow in NB medium but there was very slight amylase production without starch supplementation. With 0.5% starch there was 1.6 mg ml^{-1} maltose liberation, and it was 2 mg ml^{-1} with 1 and 2% starch. Increasing the starch concentration to 5% did not effect the yield so much, which means using FAD4 for amylase production is cost effective.

Commercial starch sources (soluble starch, corn starch, wheat starch, and potato starch) with different concentrations were tested for amylase production. After an incubation period of 2 days, enzyme activity was measured. The most effective starch source was determined as potato starch with a maltose liberation of 1.0 mg ml^{-1}. The yield for soluble starch was 0.35 mg ml^{-1} while 0.2 mg ml^{-1} maltose liberation were observed for both corn starch and wheat starch in same conditions. Some of the Bacillus strains can produce good amount of amylase with the supplementation wheat bran [24] which is a cheap starch source. Strain FAD4 is also produce amylase with the supplementation of wheat starch. Besides, some other strains utilize potato starch for amylase production. *B. licheniformis* and *B. amyloliquefaciens* were used for maltose manufacture, and potato starch was one of their starch sources [25]. High amount of amylase production was observed also for *Anoxybacillus* sp. [26] and *A. flavithermus* SO-13 [27] with potato starch. The only *Brachybacterium* strain reported in the literature *B. paraconglomeratum* Strain LB 25 was studied with potato starch [14].

3.5. Effects of Carbon Sources on Enzyme Production

Commercial carbon and nitrogen sources are known to be effective on amylase production. Different carbon sources were used for determining the effects on amylase production. After incubation at 30 °C for 2 days, cells were harvested and supernatant was used as crude enzyme extract. Standart reaction conditions were performed for each amylase. Among various carbon sources, the best results obtained from lactose (Figure 3). 1.70 mg ml^{-1} of maltose liberation observed when lactose used in growing medium. It was 1.35 mg ml^{-1} for galactose, and 0.85 mg ml^{-1} for fructose. There was no enzyme production when glucose used as carbon source. Similar results were obtained from other bacterial strains in the literature. Supplementation of the medium with 5g l^{-1} of lactose increased the amylase production from *Bacillus amyloliquefaciens* [28]. In another study, the highest amylase production from *Anoxybacillus* sp. AH1 was observed with maltose supplementation, glucose, and lactose were also increased the enzyme production [26]. The inhibitory effect of glucose on FAD4 was also observed for *Anoxybacillus flavithermus*. Aguloglu Fincan et all., [29] determined that using glucose and sucrose in growing media decreased the amylase production from *A. flavithermus* [29].

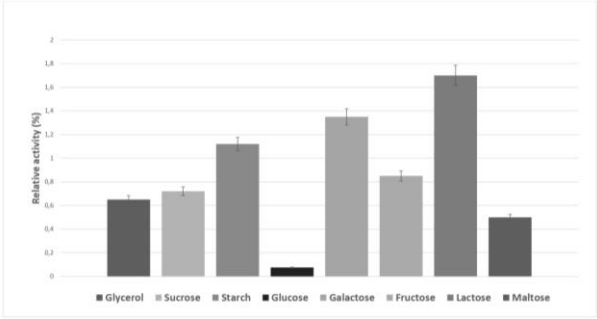

Figure 3. Effects of different carbon sources on amylase production from strain FAD4

3.6. Effects of Nitrogen Sources on Enzyme Production

Some of the nitrogen sources were also exhibited enhancing effects on amylase production from strain FAD4 (Figure 4). Among nitrogen sources used in this study peptone was the best source for enzyme production. Using 1% of peptone was increased the yield in a rate of 5.15% when compared with soluble starch, and yeast extract supplementation was also yielded a good enzyme production (only 4% lower than peptone). Other nitrogen surces decreased the enzyme production in a rate of 50% or more. Peptone and yeast extract were also suitable for the production of amylase from *Bacillus amyloliquefaciens* [28]. In another study amylase production from *Streptomyces* sp. MSC702 was induced by pepton [30]. The increasing effect of yeast extract was also observed for *Bacillus subtilis* MB6 [31] and *Bacillus* sp. [32].

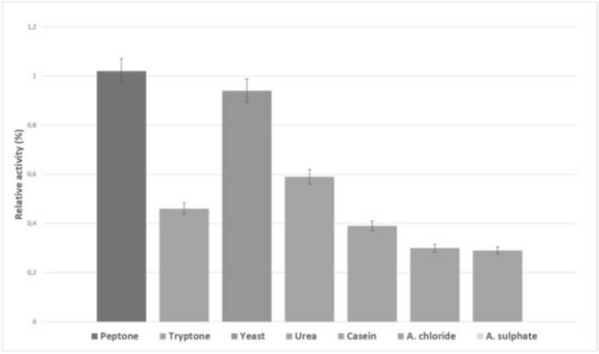

Figure 4. Effects of different nitrogen sources on amylase production from strain FAD4

4. CONCLUSION

Brachybacterium paraconglomeratum strain FAD4 was isolated from the wastewater of a textile factory located in Soke (Aydın/Turkey). FAD4 was able to hydrolyse starch with amylase production. The optimal conditions for amylase production were 72 h at 30 °C. Optimum temperature and pH of the amylase were suitable for industrial applications. A high enzyme production was observed with 1% potato starch and other starch sources (soluble, wheat, corn) were also suitable for amylase production. Among nitrogen sources peptone and yeast extract were induced the enzyme production. Lactose, galactose and fructose were also increased the enzyme production as carbon sources. The amylase production features of *B. paraconglomeratum* strain FAD4 were similar with the other amylase producing strains used in industry, and it can be said that the amylase produced by *B. paraconglomeratum* FAD4 colud be a useful tool for industrial applications.

Acknowledgement

The author is grateful to Ali Osman BELDUZ for useful discussions and suggestions.

REFERENCES

[1] Li S, Yang X, Yang S, Zhu M, Wang X. Technology prospecting on enzymes: application, marketing and engineering. Comput. Struct. Biotechnol. J. 2012;2:1–11.

[2] Gomes I, Gomes J, Steiner W. Highly thermostable amylase and pullulanase of the extreme thermophilic eubacterium *Rhodothermus marinus*: production and partial characterization. Bioresour. Technol. 2003;90 (2);2 07-214.

[3] Cantarel BL, Coutinho PM, Rancurel C, Bernard T, Lombard V, Henrissat B. The Carbohydrate-Active EnZymes database (CAZy): an expert resource for Glycogenomics. Nucleic Acids Res. 2009;37;233–238.

[4] Nagano N, Orengo C.A, Thornton JM. One fold with many functions: the evolutionary relationships between TIM barrel families based on their sequences, structures and functions. J. Mol. Biol. 2002;321;741-765.

[5] Gupta R, Gigras P, Mohapatra H, Goswami VK, Chauhan B. Microbial α-amylases: a biotechnological perspective. Process Biochem. 2003;38;1599 – 1616.

[6] Ma Y, Yang H, Chen X, Sun B, Du G, Zhou Z, et al. Significantly improving the yield of recombinant proteins in Bacillus subtilis by a novel powerful mutagenesis tool (ARTP): Alkaline α-amylase as a case study. Protein Express. Purif. 2015;114;82-88.

[7] Shafiei M, Ziaee AA, Amoozegar MA. Purification and characterization of an organic-solvent-tolerant halophilic α-amylase from the moderately halophilic Nesterenkonia sp. strain F. J. Ind. Microbiol. Biotechnol. 2011;38;275–81.

[8] Hussain I, Siddique F, Mahmood MS, Ahmed SI. A Review of the Microbiological Aspect of α-amylase Production. IJAB. 2013;15(5);1029-1034.

[9] Sundarram A, Murthy TPK. α-Amylase production and applications: a review. J. Appl. Environ. Microbiol. 2014; 2;166-175.

[10] Collins MD, Brown J, Jones D. Brachybacterium faeciumgen. nov., sp. nov., a coryneform bacterium from poultry litter. Int. J. Syst. Bacteriol. 1988;38; 45–48.

[11] Lapidus A, Pukall R, Labuttii K, Copeland A, Glavina Del Rio T, Nolan M, et al. Complete genome sequence of *Brachybacterium faecium* type strain (Schefferle 6-10). *Stand. Genomic Sci*. 2009;1;3–11.

[12] Park SK, Kim MS, Jung MJ, Nam YD, Park EJ, Roh SW. et al. *Brachybacterium squillarum* sp. nov., isolated from salt-fermented seafood. *Int. J. Syst. Evol. Microbiol.* 2011;61;1118–1122.

[13] Singh H, Du J, Yang JE, Yin CS, Kook M, Yi TH. *Brachybacterium horti* sp. nov., isolated from garden soil. *Int. J. Syst. Evol. Microbiol.* 2016;66;189–195.

[14] Doukyu N, Yamagishi W, Kuwahara H, Ogino H, Furuki N. Purification and characterization of a maltooligosaccharide-forming amylase that improves product selectivity in water-miscible organic solvents, from dimethylsulfoxide-tolerant Brachybacterium sp. strain LB25. Extremophiles. 2007;11;781–788.

[15] Atlas RM, Parks LC, Brown AE. Laboratory Manual of Experimental Microbiology. St Louis: Mosby-Year Book Inc.; 1995.

[16] Bernfeld P. Amylases, *α* and *β*. Methods. Enzymol. 1955;1;149–158.

[17] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 1976;72;248-254.

[18] Mageswari A, Subramanian P, Chandrasekaran S, Sivashanmugam K, Babu S, Gothandam KM. Optimization and immobilization of amylase obtained from halotolerant bacteria isolated from solar salterns. J. Genet. Eng. Biotechnol. 2012;10(2);201-8.

[19] Konsoula Z, Liakopoulou-Kyriakides M. Co-production of alpha-amylase and beta-galactosidase

by *Bacillus subtilis* in complex organic substrates. Bioresour. Technol. 2007;98;150–157.
[20] Souza PMD, Pérola OM. Application of Microbial α-Amylase in Industry-A Review. Braz. J. Microbiol. 2010;41;850-861.
[21] Doukyu N, Yamagishi W, Kuwahara H, Ogino H. A Maltooligosaccharide-Forming Amylase Gene from Brachybacterium sp. Strain LB25: Cloning and Expression in Escherichia coli. Biosci. Biotechnol. Biochem. 2008;72(9);2444–2447.
[22] Farooq MA., Ali S, Hassan A, Tahir HM, Mumtaz S, Mumtaz S. Biosynthesis and industrial applications of α-amylase: a review. Arch. Microbiol. 2021;203;1281–1292.
[23] Mehta D, Satyanarayana T. Bacterial and archaeal α-amylases: diversity and amelioration of the desirable characteristics for industrial applications. Front. Microbiol. 2016;7;1129.
[24] Yildirim Akatın M. An Overview of Amylase Production by Solid State Fermentation (SSF) since 2010. JTST. 2019;9(1);1-7.
[25] Aiyer PV. Amylases and their applications. Afr. J. Biotechnol. 2005;4 (13);1525-1529.
[26] Acer O, Pirinccioglu H, Bekler FM, Gul-Guven R, Güven K. Anoxybacillus sp. AH1, an α-amylase-producing thermophilic bacterium isolated from Dargecit Hot Spring. Biologia. 2015;70(7);853-862.
[27] Ozdemir S, Okumus V, Ulutas MS, Dundar A, Akarsubası AT, et al. Isolation of a Novel Thermophilic *Anoxybacillus flavithermus* SO-13, Production, Characterization and Industrial Applications of its Thermostable α-Amylase. J. Bioprocess Biotech. 2015;5;237.
[28] Sharma N, Vamil R, Ahmad S, Agarwal R. Effect of Different Carbon and Nitrogen Sources on A-Amylase Production from *Bacıllus Amyloliquefacıens.* IJPSR. 2012;3(4);1161-1163.
[29] Fincan SA, Enez B, Ozdemir S, Bekler FM. Purification and characterization of thermostable a-amylase from thermophilic Anoxybacillus flavithermus. Carbohydr. Polym. 2014;102;144-150.
[30] Singh R, Kapoor V, Kumar V. Influence of Carbon and Nitrogen Sources on the α-amylase Production by a Newly Isolated thermophilic Streptomyces sp. MSC702 (MTCC 10772). Asian J. Biotechnol. 2011;3;540-553.
[31] Lall BM, Paul JS, Jadav SK, Tiwari KL. Effect of Carbon and Nitrogen Source α-Amylase Enzyme Production from *Bacillus Subtilis* MB6. Indian J. Aerobiol. 2016;29;37-41.
[32] Qader SAU, Bano S, Aman A, Syed N, Azhar AA. Enhanced productionand extracellular activity of commercially important amylolytic enzyme by anewly isolated strain of Bacillus sp. AS-1. TJB. 2006;31;135–140.