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Thermophilic and Halophilic Microorganisms Isolated from Extreme Environments of Turkey, with Potential Biotechnological Applications

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Abstract

Turkey has a great number of different ecological areas, owning over 200 hot water resources and various hypersaline environments with a broad microbial diversity and opportunities for newly isolated microorganisms from extreme environments for many industrial applications. A variety of thermophilic and halophilic microorganisms in different regions of Turkey have been isolated and identified. The thermophilic bacterial members studied were *Anoxybacillus*, *Geobacillus*, *Bacillus*, *Brevibacillus*, and *Aeribacillus* belonging to the *Bacillaceae* family and the other thermophiles such as *Thermus* and *Thermomonas*, whereas the isolated halophilic microorganisms were mainly found to be members of the archaeal family *Halobacteriaceae* or grouped into bacterial phylum *Bacteroidetes*. In summary, the present study reviews on (1) isolating and identifying thermophiles and halophiles single or as community from various extreme habitats in Turkey based on conventional (morphological, physiological and biochemical tests) and/or molecular methods, (2) screening these extremophiles for industrially important enzymes, (3) studying other novel products and their use in other areas of biotechnology, and finally (4) discussing about the development strategies and the future perspectives on poorly studied extremophilic microorganisms in the country to fulfill future biotechnological and industrial demands.

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8.1 Introduction

Microbes in nature have served one of the largest and useful sources of many bioproducts including enzymes, polymers such as exopolysaccharides and polyhydroxyalkanoate, osmolytes, etc. Recently, biotechnology has increased its efforts to search for new organisms of practical use. Many microbial taxa need to be discovered and isolated from various extreme environmental samples all over the world. Extremophiles can survive in a variety of extreme conditions, which are classified as halophiles, thermophiles, psychrophiles, alkalophiles, acidophiles, barophiles, metalophiles and others depending on their adaptations to unusual environmental conditions.

Turkey has lots of different ecological areas, which possesses a broad microbial diversity. Turkey is a peninsular country surrounded by seas and also well known for its geothermal activity, and there are so many thermal springs all over the country. Therefore, there should be a great deal of opportunities for newly isolated microorganisms from extreme environments, including thermophilic and halophilic ones with numerous biotechnological applications. Because a search of extremophiles in the country is very recent, this potential has not been fully exploited.

It has been already easy to detect novel and rare microorganisms due to the improved classification methods based on the integrated use of phenotypic and genotypic data, also thanks to the molecular techniques developed most recently to expand the search for new bioproducts by exploring the diversity of microorganisms. Microbial diversity and novel molecular techniques, like genomics and metagenomics, are being utilised to discover new microbial enzymes and other bioproducts whose properties can be modified/improved by varying strategies, as well as using different bioinformatics tools. Microbial bioproducts with potential for biotechnological applications are obtained from a variety of bacterial groups including extremophiles, as well as mesophiles. Recent advances in modern biotechnology have led to great development in new bioproducts, through bioproduct applications; mainly enzymes are already well established. The application of novel biotechnology research in environmentally friendly bioprocesses is also rapidly expanding.

For many decades, the *Bacillaceae* family members have been good sources in biotechnological processes concerning whole cells or enzymes. In Turkey, the isolated and identified thermophilic members of the *Bacillaceae* family include *Anoxybacillus*, *Geobacillus*, *Bacillus*, *Brevibacillus*, *Aeribacillus*, etc. Moreover, isolated halophilic microorganisms were mainly found to be members of both bacteria and archaea.

In this chapter, an attempt has been made to review on the diversity of microorganisms isolated and identified in extreme environments of Turkey, which are hot water resources and hypersaline salterns, salt lakes or salt mines, as well as on the potential biotechnological applications of their industrially important enzymes and other novel products.

8.2 Thermal Springs Studied in Turkey

It is well known that hot water resource and geothermal region are the main thermophilic regions. Hot water resources are located in different parts of the world, due to volcanic activities. Turkey possesses many geothermal sources with varying typical temperatures and pH values. Figure 8.1 shows the map of all thermal water resources and those studied and documented within this review. The hot springs in Turkey, where the temperatures vary from 36 to 80 °C, are mostly rich in calcium and magnesium ions. It is well known that these springs are used for curing so many neurological, gynaecological, rheumatismal and dermatological diseases, as well as for digestive disorders and physical exhaustion (http://turkeyculture.org/). Although we are going to review on the studies carried out so far on the thermophilic microorganisms within this chapter, it should be mentioned that these environments have not yet been intensively studied in terms of microbiological diversity.

8.3 Thermophilic Microorganisms Isolated and Identified in Turkey

Microbial growth requires temperature as a vital parameter, and different temperature ranges are preferred by microorganisms to survive. Thus, microorganisms are grouped as psychrophiles, mesophiles, thermophiles and hyperthermophiles.

The family *Bacillaceae* currently consists of 62 genera and known as one of the largest bacterial families. The majority of *Bacillaceae* produce endospores and are Gram-positive, either rod-shaped (bacilli) or spherical (cocci), bacteria. *Bacillus*, *Anoxybacillus*, *Geobacillus*, *Brevibacillus*, etc. are classified into *Bacillaceae*, within the phylum *Firmicutes*, class *Bacilli* and order *Bacillales* (Mandic Mulec et al. 2016). The presence of thermophilic bacteria in the *Anoxybacillus*, *Bacillus*, *Geobacillus*, *Brevibacillus*, *Thermus* and *Aeribacillus* genera in thermal areas has been reported in Turkey (Belduz et al. 2003; Gul Guven et al. 2008; Inan et al. 2012; Poli et al. 2012; Bozoglu et al. 2013; Kacagan et al. 2015; Baltaci et al. 2016; Yildirim et al. 2017).

8.3.1 Anoxybacillus

Among bacilli members, unlike *Brevibacillus* and *Bacillus*, *Anoxybacillus* is a rather new genus that was recently proposed, known as aerotolerant anaerobes or

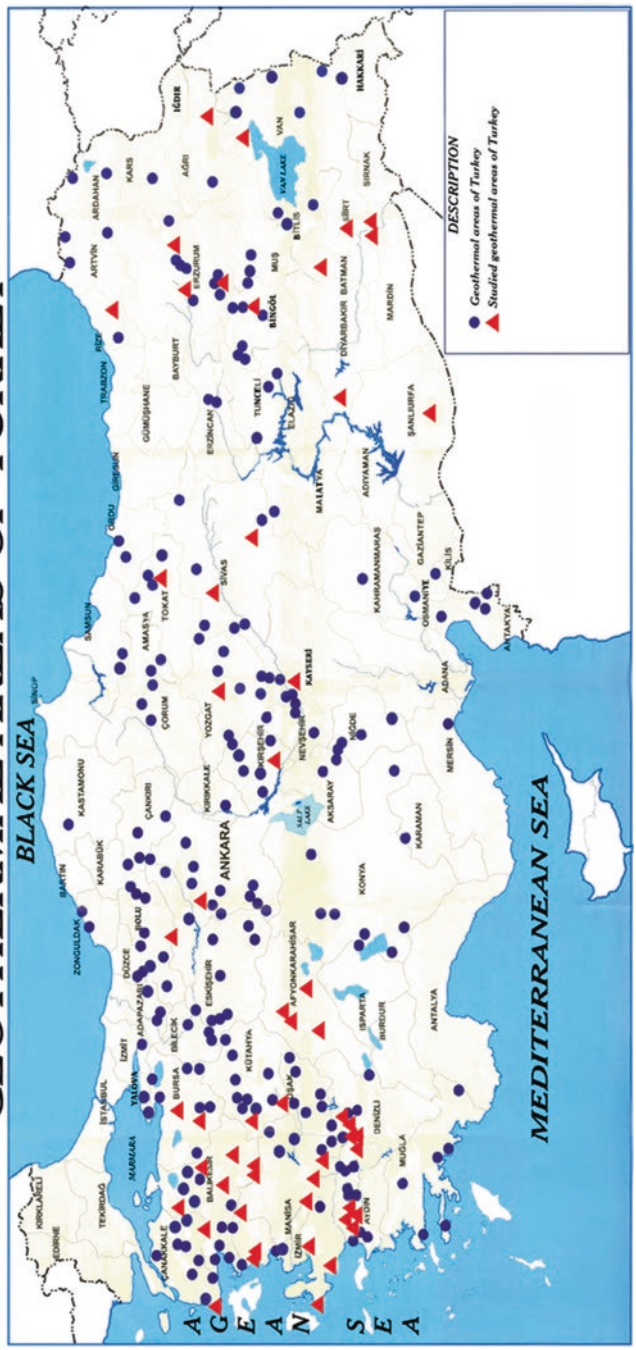

Fig. 8.1 The map of all geothermal areas in Turkey (blue circles) and those studied for microbial diversity within this review (red triangles), obtained and modified from documents of MTA (General Directorate of Mineral Research and Exploration of Turkey)

facultative anaerobes. The genus name *Anoxybacillus* means small rod living in the absence of oxygen (Pikuta et al. 2000). The members of the genus *Anoxybacillus* are Gram-positive, endospore-forming, rod-shaped (bacilli) bacteria, sizing 0.4–1.5 × 2.5–9.0 μm. Most *Anoxybacillus* species are moderately thermophilic having an optimal growth from 50 to 65 °C. *Anoxybacillus* cells are known to be alkalitolerant thermophile, which are suitable for most industrial applications. Since the first report of *Anoxybacillus*, this genus has been shown to serve as a possible choice in various applications involving lignocellulosic and starch biomasses, enzyme technology, waste treatment and also bioenergy manufacturing (Goh et al. 2013; Cihan and Yildiz 2016). As shown in Table 8.1, several important *Anoxybacillus* members have been identified, and their potential use in biotechnology has been evaluated in Turkey. In this section, the review of relevant literature will be presented in the chronological order.

Belduz et al. (2003) studied seven xylanolytic, thermophilic bacterial strains from mud and water samples from two major hot springs, namely, Gonen and Diyadin, located in the Turkish provinces of Balikesir and Agri, respectively. Among these strains, following morphological, biochemical and genetic analysis, *Anoxybacillus gonensis* was found to be a novel sporulating, rod-shaped, thermophilic bacterium (with an optimum temperature of 55–60 °C), growing on various carbon sources, such as xylose, glucose, starch and mannitol. It was also found to produce a high level of xylose isomerase.

Two moderately thermophilic (optimum temperature for growth, 50–55 °C) *Anoxybacillus* species were also isolated from Kestanbol and Ayder hot springs in Canakkale and Rize provinces, respectively, by Dulger et al. (2004). They were identified as *A. ayderensis* and *A. kestanbolensis* which were sporulating, Gram-positive, rod-shaped bacteria, growing on a variety of carbon sources including maltose, D-sucrose, D-glucose, D-mannose, D-mannitol, D-raffinose, D-fructose, D-xylose and L-arabinose.

In addition, Gul Guven et al. (2008) isolated a novel thermophilic Gram-positive strain KG8$^{(T)}$ from Taslidere hot spring in Batman. This strain was motile, spore-forming, aerobe, rod-shaped and occurring in pairs or filamentous. The growth range was between 35–65 °C (optimum temperature of 55 °C) and at pH 5.5–9.5 (optimum pH of 7.5). Because the strain KG8 was incapable to utilise most carbohydrates, this new subspecies was named as *A. kamchatkensis* subsp. *asaccharedens*. 16S rRNA gene sequence similarity, chemotaxonomic data and the results of biochemical and physiological tests, DNA–DNA hybridisation allowed phenotypic and genotypic differentiation of strain KG8 supporting the affiliation to the genus *Anoxybacillus*. This subspecies was found to be a good source of the enzyme amylase capable of utilising starch.

In a microbial diversity study, Adiguzel et al. (2009) identified 15 Gram-positive thermophilic bacteria isolated from Pamukcu (Balikesir), Sorgun (Yozgat), Ilica and Akdag (Erzurum) hot springs by using various methods including phenotypic, chemotaxonomic, 16S rRNA sequencing and rep-PCR genomic fingerprint profilings. They suggested that these profilings can be used as a reliable technique to identify thermophilic bacteria in the genera of *Bacillus*, *Anoxybacillus* and *Geobacillus* spp.

Table 8.1 *Anoxybacillus* members isolated and identified from various regions of Turkey and their potential use in biotechnology

Microorganisms	Strain code	GenBank accession number	Source	Growth temperature (°C)	Growth pH	Biotechnological applications and products	Reference
Anoxybacillus ayderensis	AB04^T	NCIMB 13972^T	Ayder hot spring/Rize	50–55	7.5–8.5	Xylose isomerase, esterase, fructose-1,6-bisphosphate aldolase, heavy-metal resistance	Dulger et al. (2004) and Belduz et al. (2015)
Anoxybacillus calidus	C161ab^T	FJ430012	Kizildere hot spring/Denizli	55	8.0-8.5	Amylase	Cihan et al. (2014)
Anoxybacillus flavithermus	M14, VI, R32, R40, M6.p1, 75B	–	Balcova geothermal site/ Izmir	30–70	9.0–10.0	Amylase, lipase	Yavuz et al. (2004)
Anoxybacillus flavithermus	–	–	Omer hot spring/ Afyonkarahisar	55	7.0	Amylase, metal biosorption	Ozdemir et al. (2011), Fincan et al. (2014) and Yener et al. (2017)
Anoxybacillus flavithermus	–	–	Gazligol hot spring/ Afyonkarahisar	60	7.0	Amylase	Ozdemir et al. (2012)

Anoxybacillus gonensis	G2^{T}	NCIMB 13933^{T}	Gonen hot spring/Balikesir	55–60	7.5–8.0	Xylose-utilising, esterase, thermozyme, amylase, pullulanase, cyclodextrinase, β-xylosidase, xylose isomerase, second-generation biofuel production, oligo-1,6-glucosidase, fructose-1,6-bisphosphate aldolase, α-galactosidase, α-glucosidase, β-galactosidase, mercury and arsenate reductase, glucose isomerase, CTP synthase, heavy-metal biosorption, ATPase activity of aluminium tolerance protein	Belduz et al. (2003), Colak et al. (2005), Faiz et al. (2007), Ertunga et al. (2007), Duran et al. (2009), Beris et al. (2011), Oztekin et al. (2011), Karaoglu et al. (2013), Sandalli et al. (2014), Lim et al. (2015) and Belduz et al. (2015)
Anoxybacillus gonensis	P39-89	FJ808725-26	Ilica hot spring/Erzurum	55	7.5-10.0	Removal of textile dye, laccase	Adiguzel et al. (2009) and Yanmis et al. (2016)
Anoxybacillus gonensis	Z4		Ilica hot spring/Erzurum	55	7.2	Amylase	Baltas et al. (2012)
Anoxybacillus gonensis	A4-O9	AY248707 KM596794	Diyadin hot spring/Agri	60	7.5	Amylase, cellulase	Colak et al. (2008), Genc et al. (2014), (2015) and Baltaci et al. (2016)
Anoxybacillus kamchatkensis subsp. *asaccharedens*	KG8$^{(T)}$	AM999779	Taslidere hot spring/Batman	55	7.5	Amylase	Gul Guven et al. (2008)

(continued)

Table 8.1 (continued)

Microorganisms	Strain code	GenBank accession number	Source	Growth temperature (°C)	Growth pH	Biotechnological applications and products	Reference
Anoxybacillus kaynarcensis	D1021^T	EU926955	Kaynarca hot spring/İzmir	60	7.0	Xylanase	Inan et al. (2013)
Anoxybacillus kaynarcensis	A1, A13	KC310452 KC310464	NED[1]	57±1	6.5–9.0	Amylase, cellulase	Yanmis et al. (2015)
Anoxybacillus flavithermus	A2	KC310453				Amylase	
Anoxybacillus rupiensis	A3	KC310454				Amylase	
Anoxybacillus gonensis	A5	KC310456				Amylase, cellulase	
Anoxybacillus kestanbolensis	K4^T	NCIMB 13971^T	Kestanbol hot spring/ Canakkale	50–55	7.5–8.5	Ribulokinase, protease	Dulger et al. (2004), Atasoy et al. (2012) and Tokgoz et al. (2014)
Anoxybacillus pushchinoensis	A8	AY248715	Diyadin hot spring/Agri	60	7.0–8.8	Xylanase	Kacagan et al. (2008)
Anoxybacillus salavatliensis	A343^T	EU326496	Salavatli hot spring/Aydin	60	8.0–9.0	α-Glucosidase	Cihan et al. (2011a)
Anoxybacillus sp.	PDF1	HQ875718	Seferihisar and Karakoc hot springs/Izmir	55	7.4	Carboxylesterase	Ay et al. (2011)
Anoxybacillus sp.	KP1	KC525949	Kopru hot spring/Agri	50	8.0	Amylase, protease, β-galactosidase	Matpan Bekler and Guven (2014) and Matpan Bekler et al. (2015c, 2017)
Anoxybacillus sp.	AH1	KP172526	Dargecit hot spring/Mardin	60	7.0–7.5	Amylase	Acer et al. (2015, 2016)

Anoxybacillus sp.	HBB 16 HBB 134	KR91195 GQ342689	Alangullu hot spring/Aydin	65	6.5–8.0	Lipase	Bakir and Metin (2015, 2017)
Anoxybacillus sp.	KB4	KU997674	Kusburnu hot spring/Agri	55–60	9.0–10.0	Amylase	Matpan Bekler (2016)
Anoxybacillus sp.	O20	KM668039	NED[2]	56	7.0	Amylase, cellulase, lipase	Genc et al. (2015) and Baltaci et al. (2016)
Anoxybacillus sp.	G18	FJ808715	Sorgun hot spring/Yozgat	55–60	7.5–10.0	–	Adiguzel et al. (2009)
Anoxybacillus thermarum	A4	KC310455	Erzurum	55	7.0	Amylase, cellulase	Yanmis et al. (2015) and Baltas et al. (2016)
Anoxybacillus sp.	NB	FJ215763	Hisaralan hot spring/Balikesir	70	7.5	DNA polymerase I	Caglayan and Bilgin (2011)

NED: The samples collected from various hot springs and the source are **not exactly defined**

NED[1]: The samples collected from Kirsehir–Terma, Sirnak/Guclu Konak–Belkis Ana, Agri–Diyadin, Kayseri–Haciveli, Erzurum–Ilica, Urfa–Karaali, Rize–Ayder, Adiyaman–Kurucay and Ankara–Kizilcahamam

NED[2]: The samples collected from Bademli Bahce hot spring (Bursa), Sulusaray hot spring (Tokat), Sicak Cermik hot spring (Sivas), Bostanci hot spring (Balikesir) and Diyadin hot spring (Agri)

The results demonstrated that thermophilic bacterial strains collected from the hot springs were classified into three main clusters, one of which consisted three *Anoxybacillus* strains.

Cihan et al. (2011a) identified a moderate thermophilic bacilli, spore-forming, Gram stain-positive, facultative anaerobic, motile and α-glucosidase-producing novel *Anoxybacillus* species named *A. salavatliensis*, obtained from a high-temperature well-pipeline sediment in Salavatli town of Aydin, Turkey. In this study, the rep-PCR (BOX-PCR, (GTG)5-PCR) and ITS fingerprinting analyses were carried out between phylogenetically related species clustering of strain A343^T with its closely related *Anoxybacillus* species, as well as performing sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS–PAGE) total protein profiles of relevant species. Growth of *A. salavatliensis* was observed at a range of 37–69 °C (optimum temperature of 60 °C) at a range of pH 5.5–9.5 (with optimum of 8.0–9.0) and able to grow on a variety of carbon sources. The biochemical tests showed the biotechnological potential of this bacterium concerning its enzymes, e.g. starch and gelatin utilisation; catalase, oxidase and amylase activities; and reduction of nitrate.

Nine of xylanolytic thermophilic microorganisms isolated from some hot springs located in the west of Turkey were found to belong to the genus *Anoxybacillus* on the basis of phenotypic characteristics and 16S rRNA gene sequence analysis (Inan et al. 2011a). Among these strains, a novel moderately thermophilic bacilli, endospore-forming, Gram-positive, motile, alkalitolerant strain D1021^T was described by Inan et al. (2013) from Kaynarca hot spring (water temperature, 60–100 °C) in Izmir Province of Turkey. The growth characteristics observed were temperature range of 35–70 °C (optimum 60 °C) and pH range of 6.0–10.0 (optimum pH of 7.0). The strain utilised a variety of carbon sources such as glucose, ribose, xylose, arabinose, maltose, melibiose and sucrose. They identified the strain as a new species and named *A. kaynarcensis* on the basis of phenotypic characteristics, phylogenetic and DNA–DNA hybridisation data, as well as *rpoB* gene analysis. In addition to 16S rRNA gene, the *rpoB* gene was shown to be successfully used as a genotyping approach to phylogenetic studies within *Anoxybacillus* (Inan et al. 2011b). The analysis of *rpoB* gene that is for the RNA polymerase beta subunit has been previously suggested in taxonomic studies of bacteria as an alternative of the 16S rRNA gene, containing conserved and variable regions (Da Mota et al. 2004).

Cihan (2013) has also studied 115 endospore-forming bacilli isolated from geothermal areas in Turkey by analysing 16S rDNA sequence analyses, as well as by ARDRA, ITS-PCR and rep-PCR. The isolates used in this study were collected from water, sediment, soil, stone and tree branch samples within ten hot springs and nine well pipelines of high temperatures, located in Aegean Region and Middle Anatolian Region. Among these strains, most widely distributed thermophiles belonged to the genus *Anoxybacillus* with 53 isolates. The isolated strains were grouped into eight phylogenetic lineages within the type strains of *A. flavithermus*, *A. salavatliensis*, *A. kamchatkensis* and *A. kamchatkensis* subsp. *asaccharedens*. In this study, the author also underlines the importance of *Anoxybacillus* isolates which produce biotechnologically valuable enzymes, the ability of carbohydrate

degradation mainly amylolytic, glucosidic and proteolytic activities that made them superior in comparison to the remaining bacilli in the extreme habitats.

A novel amylase-producing thermophilic bacterial strain KP1 from the hot spring of Diyadin in Agri, Turkey, was isolated by Matpan Bekler and Guven (2014). The strain KP1 belonged to the genus *Anoxybacillus* on the basis of phylogenetic analysis by the sequence similarity of 16S rRNA gene by biochemical and physiological tests. Moreover, a thermophilic, starch-hydrolysing bacterium identified as *A. calidus* was isolated from soil near a thermal power plant near Kizildere (the water temperature of this geothermal region is between 195 and 212 °C) within Denizli province in Aegean Region of Turkey by Cihan et al. (2014). They also analysed the strain further by using the results of rep-PCR and ITS fingerprinting differentiating from related species of the genus *Anoxybacillus*. This novel species is facultatively anaerobic, rod-shaped, Gram-positive staining, motile and endospore-forming bacterium, which grows at a temperature range between 35 and 70 °C (with optimum 55 °C), at pH range of 6.5–9.0 (with optimum 8.0–8.5).

In another study, Acer et al. (2015) isolated α-amylase-producing thermophilic bacteria from the mud of Dargecit hot spring (water temperature and pH as 58 °C and 6.9, respectively) in Mardin Province of Turkey. The isolated strain AH1 was found to be a member of *Anoxybacillus* genus by characterising with the morphological, biochemical and physiological tests, in addition to the genetic analysis by 16S rRNA sequences. The analysis of 16S rRNA gene sequence showed that the most sequence similarity of the strain AH1 (DSM 23210^T) was to *A. flavithermus* subsp. *flavithermus* DSM 2641^T by 98.23%. The strain was Gram-positive, aerobe and spore-forming rod, which had an optimum growth temperature and pH values of 60 °C and 7.0–7.5, respectively. However, it was found to grow in a wide pH range (5.5–10.0), indicating that *Anoxybacillus* AH1 cells were alkaliphilic or alkalitolerant.

Belduz et al. (2015) have recently completed the study on genome sequences of thermophilic *A. ayderensis* AB04^T (=NCCB 100050^T = NCIMB 13972^T) which was isolated and described previously from the hot spring of Ayder in Rize Province of Turkey. The strain genome was 2,832,347 bp long and found to contain 2895 predicted genes as well as 103 RNA genes including 88 tRNAs, 14 rRNAs and 1 tmRNA. *A. gonensis* type strain G2^T (=NCCB 100040^T = NCIMB 13,933^T) isolated from Gonen hot springs in Turkey and identified previously was also studied by Lim et al. (2015) for its annotated and complete genome sequencing. It was presented that the total length of the genome was 2,803,668 bp, with 41.7% G + C content. Moreover, the genome comprised of 2934 protein-coding sequences, 62 pseudogenes, 2769 CDS, 78 tRNAs and 24 rRNAs.

An aerobic, motile, rod-shaped and Gram-positive thermophilic strain KB4 was isolated by Matpan Bekler (2016) from Kusburnu hot spring (the temperature and pH of the water 70 °C and 7.5) in Agri Province of Turkey. The strain growth was obtained at temperature of 55–60 °C, at pH 9.0–10.0 and at 3% (w/v) NaCl. The sequence analysis of 16S rRNA gene indicated that the KB4 strain was closely related to *A. pushchinoensis* K1$^{(T)}$ with a sequence similarity of 98.78%.

Most recently, Baltaci et al. (2016) have studied on the identification thermophilic bacteria from the water and sludge samples of different hot springs, including Sulusaray (50 °C, pH 7.2), Sicak Cermik (53 °C, pH 6.6), Bostanci (51 °C, pH 7.2), Bademli Bahce (58 °C, pH 6.6) and Diyadin (70 °C, pH 6.8). The identification was carried out by chemotaxonomic data from FAMEs, the biochemical, physiological and morphological tests and molecular methods (16S rRNA sequencing and GTG5-PCR). The strains were resembled mainly to three genera, namely, *Anoxybacillus*, *Bacillus* and *Aeribacillus*, according to 16S rRNA sequencing results. However, a strain designated as O20 exhibited 97% similarity to *A. kaynarcensis*, while the other (O9) was resembled to *Anoxybacillus gonensis* with a similarity rate of 99%.

8.3.2 Geobacillus

Considering many bacilli genus, similar to *Anoxybacillus*, *Geobacillus* is also a relatively new genus that was recently proposed by Nazina et al. (2001), of which members are thermophilic, endospore-forming and aerobic, grow at a temperature range between 35 and 78 °C and are also widespread in many geographical areas (Poli et al. 2011; Cihan et al. 2011b).

There have been some reports on members of *Geobacillus* genus isolated from hot springs within Turkey and on their industrially important enzymes and new products (Table 8.2). The first report was carried out by Canakci et al. 2007 a decade ago. They isolated 16 Gram-positive rods from water and mud samples of Dikili–Bergama Kaynarca hot spring in province of Izmir and Camkoy Camur, Omerbeyli and Alangullu hot springs in the province of Aydin. The water temperatures of the hot springs studied varied between 70 and 130 °C. Based on 16S rRNA gene sequence analysis, all of 16 isolates resembled *Geobacillus* species by ≥97%, and most of them were found to produce xylanase and arabinofuranosidase enzymes.

Adiguzel et al. (2009) also studied as many as 15 thermophilic bacilli isolated from several different hot springs in Turkey and then characterised and identified by using molecular methods such as (GTG) 5-PCR cluster analysis. *G. pallidus* subcluster was comprised of the strains G19A, P112, P66 and P161 with similarity ratios of ≥82%, ≥80% and ≥ 81%, respectively. Second cluster included four strains of *G. toebii* and *G. stearothermophilus* (M66A, Ah22, G5A and G7 and a reference strain of *G. thermodenitrificans*), with lower similarity ratio (≥76%).

In a very comprehensive study, Coleri et al. (2009) isolated 451 thermophilic bacilli from 42 different hot springs and high-temperature power plants of different locations in the provinces of Ankara, Denizli, Aydin, Manisa, Nevsehir and Izmir belonging to different geographical regions of Turkey. The water temperatures of these geothermal waters were in the range 60–90 °C and pH of 6.0–9.0. Sixty-seven isolates showed a high amylase activity. All isolates were Gram-positive, rod-shaped, endospore-forming, motile, catalase-positive bacteria. Four thermophilic bacilli strains, F84b, A333, F84a and E134, producing α-glucosidase at significant levels were chosen for further experiments. The 16S rRNA gene sequence analysis showed that all isolates chosen belonged to the genus *Geobacillus*. *Geobacillus* spp.

Table 8.2 *Geobacillus* members isolated and identified from various regions of Turkey and their potential use in biotechnology

Microorganisms	Strain code	GenBank Accession number	Source	Growth temperature (°C)	Growth pH	Biotechnological applications and products	Reference
Geobacillus anatolicus	C4	AF411064	Hisaralan hot spring/Balikesir	70	7.5	DNA polymerase I	Caglayan and Bilgin (2012)
Geobacillus bogazici	NT	AY323205	Hisaralan hot spring/Balikesir	70	7.5	DNA polymerase I	Caglayan and Bilgin (2011)
Geobacillus kaue	NB	AF411066	Gonen hot spring/ Balikesir	70	7.5	DNA polymerase I	Caglayan and Bilgin (2011)
Geobacillus pallidus	G19A	FJ808716	Sorgun hot spring/Yozgat	55–60	7.5–10.0	–	Adiguzel et al. (2009)
	P66, P112	FJ808718, FJ808721	Akdag hot spring/ Erzurum				
Geobacillus pallidus	AH23, M21, G19 P26-45	EU9355598, EU9355594, EU9355595, EU9355591-93	Pasinler hot spring/Erzurum	55	7.5–8.5	Amylase	Adiguzel et al. (2011)
Geobacillus sp.	R30, R72, R21, V90		Balcova geothermal site/ Izmir	30–70	9.0–10.0	Amylase, lipase	Yavuz et al. (2004)
Geobacillus sp.	DB2	KX184730	Dibekli hot spring/Agri	60	6.0–6.5	Amylase	Matpan Bekler (2017)
Geobacillus sp.	TF16	KY013619	Germencik hot spring/Aydin	55		Endo-xylanase, phytase	Cakmak and Saglam Ertunga (2017) and Sirin et al. (2017)
Geobacillus sp.	P161	FJ808724	Ilica hot spring/ Erzurum	55–60	7.5–10.0		Adiguzel et al. (2009)

(continued)

Table 8.2 (continued)

Microorganisms	Strain code	GenBank Accession number	Source	Growth temperature (°C)	Growth pH	Biotechnological applications and products	Reference
Geobacillus spp.	I2, IT4.1 11, IT3, 9.1, 4.5, I4.3		NED[1]	55	5.5-8.5	Xylanase, arabinofuranosidase	Canakci et al. (2007)
Geobacillus stearothermophilus	AH22	FJ808712	Ilica hot spring/ Erzurum	55	7.2	Lipase	Adiguzel et al. (2009) and Ekinci et al. (2016)
Geobacillus subterraneus subsp. *aromaticivorans*	Ge1[T]	CIP 110341[T]	Guclukonak hot spring/Sirnak	60	9.0	β-Galactosidase, lipase	Poli et al. (2012)
Geobacillus thermodenitrificans	A333	EU326497	Germencik hot spring/Aydin	60	6.8	α-Glucosidase	Cihan et al. (2009)
Geobacillus thermodenitrificans subsp. *calidus*	F84b[(T)]	NCIMB 14582[(T)]	Kizilcahamam hot spring/Ankara	60	8.0	α-Glucosidase	Cihan et al. (2011b)
Geobacillus toebii	G5A, G7	FJ808713, FJ808714	Sorgun hot spring/Yozgat	55–60	7.5–10.0	–	Adiguzel et al. (2009)
	M66A	FJ808717	Pamukcu hot spring/Balikesir				

NED: The samples collected from various hot springs and the source is **not exactly defined**
NED[1] : The samples collected from Dikili–Bergama Kaynarca hot spring and Camkoy Camur, Omerbeyli, and Alangullu hot springs in the provinces of Izmir and Aydin in Turkey

F84a, A333 and F84b strains were determined as extracellular enzyme producers. Moreover, seven thermophilic *Geobacillus* strains were isolated from the hot springs named Hisaralan and Gonen in Turkey by Caglayan and Bilgin (2011) to study novel DNA polymerases. They cloned and sequenced the complete coding sequences of the polA genes (2637 bp) of these *Geobacillus* species, encoding DNA polymerase I with a molecular weight of 99 kDa.

Cihan et al. (2011b) also isolated a thermophilic, endospore-forming, facultatively anaerobic, rod-shaped and motile bacterial strain F84b$^{(T)}$ from well-pipeline sediment sample with a high temperature in Kizilcahamam, Turkey. The growth was observed at temperatures between 45 and 69 °C (optimum 60 °C) and pH ranging of 7.0–8.5 (optimum 8.0). Strain F84b$^{(T)}$ was found to produce α-glucosidase, growing on various carbon sources. The G + C content of genomic DNA was 49.6 mol %. The 16S rRNA gene sequence analysis of the strain F84b$^{(T)}$ displayed a high relatedness to *G. subterraneus* (99.3%) and to *G. thermodenitrificans* (99.8%) with DNA hybridisation values of 29.1% and 74.3%, respectively. In this study, rep-PCR and the intergenic 16S–23S rRNA gene fingerprinting profiles as well as the physiological and biochemical methods helped to differentiate strain F84b$^{(T)}$ from *G. thermodenitrificans*. For this reason, F84b$^{(T)}$ strain is assigned as a new subspecies and named *G. thermodenitrificans* subsp. *calidus* (=NCIMB 14582$^{(T)}$ = DSM 22629$^{(T)}$).

In a more recent study, a new thermophilic rod-shaped, endospore-forming, Gram-positive, alkaliphilic and motile *Geobacillus* strain was isolated from the mud of Guclukonak hot spring in Sirnak city in the southeast region of Turkey (Poli et al. 2012). The temperature and pH of the muddy water of the hot spring were 60 °C and 6.9, respectively. Growth of the isolate was observed at the temperature range of 30–65 °C (with optimum of 60 °C) and at pH range between 5.5 and 10.0 (optimum pH of 9.0). The strain was able to utilise starch and gelatine, the ONPG activity, positive for lipase, phosphatase, catalase and urease. 16S rRNA gene sequence studies in comparison to other members showed that the isolate belonged to the genus *Geobacillus*. The genomic DNA G + C content of the strain was 52.0%. The DNA–DNA hybridisation results showed that the representative strain Ge1^{T} closely related to *G. subterraneus*, *G. thermodenitrificans*, *G. thermocatenulatus*, *G. vulcani* and *G. thermoleovorans* were 69.3%, 57%, 37%, 27% and 26%, respectively. Chemotaxonomic analyses of FAME and other conventional tests allowed phenotypic and genotypic differentiation of this strain to be assigned as a novel subspecies named as *G. subterraneus* subsp. *aromaticivorans* Ge1^{T} (DSM 23066 ^{T} = CIP 110341^{T}), due to utilising typical hydrocarbons such as n-decane and squalene. RAPD–PCR using both OPR2 and GTG5 primers is also used for comparison, and the fingerprint profiles of the strain were clearly different from those produced by its closest relatives as well as *G. subterraneus*.

8.3.3 *Bacillus*, *Brevibacillus*, *Aeribacillus* and the Other Thermophiles

Literature review showed that there have been a few other species belonging to *Bacillus*, *Brevibacillus*, *Aeribacillus* and *Thermus* genus isolated from the thermal waters in different areas of Turkey and studied for their biotechnological potential (Table 8.3). Among these, *Brevibacillus* is reclassified from *Bacillus brevis* (first described in 1900) more recently as the type species (*Brevibacillus brevis*) of a new genus (Shida et al. 1996; Inan et al. 2012, 2016).

Members of *Bacillus* genus are well known to be widespread all over the world in various extreme and geographical areas including hot springs of Turkey. Adiguzel et al. (2009) studied several hot springs in different provinces of Turkey isolating 15 thermophilic bacteria and observed three clusters containing *Anoxybacillus*, *Geobacillus* and *Bacillus* strains by classification using 16S rRNA sequences and rep-PCR profiling techniques and showed that P151, P100, P79 and P130 strains were resembled to strains of *B. licheniformis* and *B. pumilus*.

A thermostable metalloprotease-producing bacterial strain KG5 was isolated from the mud of Kos hot spring in Bingol. The strain KG5 which was facultative anaerobic, motile, rod-shaped and Gram-positive and possessing central and oval endospores, grown optimally at 40 °C, was found to be a strain of *B. cereus* defined by phenotypic characterisation and by gene sequence analysis of 16S rRNA (Gul Guven 2007; Ahmetoglu et al. 2015). In another study carried out in a hot spring named Taslidere (water temperature of 78 °C) in Batman located in the southeast of Turkey, a thermotolerant rod-shaped bacterial strain was isolated and deposited as DSM 18503. The growth temperature range of this thermo-alkalitolerant strain KG9 was determined as 30–55 °C and pH of 5.0–10.5. The isolate was found to be a member of the species *B. licheniformis* identified by the analysis of morphological, biochemical and physiological characteristics, as well as by 16S rRNA gene sequence similarities. It was revealed that the 16S rRNA gene sequence of the strain KG9 was 99.9% similar to that of *B. licheniformis* strain DSM 13 (Gul Guven 2007; Matpan Bekler et al. 2015a). Matpan Bekler et al. (2015b) also studied on the isolation, identification and enzyme production of the strain *B. licheniformis* DV3, which were isolated from the water of Davut hot spring in Diyadin township of Agri Province (water temperature 78 °C, pH 7.7), in northeastern Turkey. The strain *B. licheniformis* DV3 was identified by biochemical, morphological tests and 16S rRNA sequence analysis.

Additionally, Adiguzel et al. (2011) revealed a population of *B. licheniformis* and *Aeribacillus pallidus* in Pasinler hot spring, Erzurum Province in Turkey, carrying out a study using the analyses of 16S rRNA gene sequences, FAME and BOX PCR fingerprint profiles. Nine different bacterial strains selected based on biochemical, physiological and morphological tests were classified into two phenotypic groups; the first group represented by four strains was identified as *B. licheniformis*, while the second group represented by five strains was identified as *A. pallidus*. In a similar study, more than ten strains were isolated from different geothermal areas of Turkey, five of which were found to belong to *A. pallidus*, six strains were close to

Table 8.3 The members of *Bacillus*, *Brevibacillus*, *Aeribacillus* and the other thermophiles isolated and identified and their potential use in biotechnology, from various regions of Turkey

Microorganisms	Strain code	GenBank accession number	Source	Growth temperature (°C)	Growth pH	Biotechnological applications and products	Reference
Bacillus licheniformis	A1	EU314720	Diyadin hot spring/Agri	60	7.0	Chitinase	Sandalli et al. (2008)
Bacillus licheniformis	G3B, G1, P38, AH2	EU9355596, EU9355597, EU9355592, EU9355599	Pasinler hot spring/ Erzurum	55	7.5–8.5	Amylase	Adiguzel et al. (2011)
Bacillus licheniformis	A10	KC310461	Ilica hot spring/ Erzurum	57	6.5–9.0	Amylase, protease, cellulase	Yilmaz et al. (2014) and Yanmis et al. (2015)
Bacillus licheniformis	A7, A9	KC310458, KC310460,	NED[1]	57±1	6.5-9.0	Amylase, cellulase	Yanmis et al. (2015)
Bacillus licheniformis	O12, O16	KM596797, KM596801	NED[2]	56	7.0	Amylase, protease, lipase, cellulase	Baltaci et al. (2016)
Bacillus licheniformis	P79	FJ808719	Akdag hot spring/ Erzurum	55–60	7.5–10.0	–	Adiguzel et al. (2009)
	P100, P151	FJ808720, FJ808723	Pamukcu hot spring/ Balikesir				
Bacillus licheniformis	KG9	DSM 18503	Taslidere hot spring/ Batman	30–55	5.0–10.5	β-Galactosidase, heavy-metal removal	Gul Guven, (2007), Matpan Bekler et al. (2015a) and Alkan et al. (2015)

(continued)

Table 8.3 (continued)

Microorganisms	Strain code	GenBank accession number	Source	Growth temperature (°C)	Growth pH	Biotechnological applications and products	Reference
Bacillus licheniformis	DV3	HQ864575	Davut hot spring/Agri	55	7.0	Amylase, protease	Matpan Bekler et al. (2015b)
Bacillus pumilus	P130	FJ808722	Pamukcu hot spring/ Balikesir	55–60	7.5–10.0		Adiguzel et al. (2009)
Bacillus pumilus	O2, O7, O8, O14	KM596787, KM596792, KM596793, KM596799	NED[2]	56	7.0	Amylase, protease, lipase, cellulase	Baltaci et al. (2016)
Bacillus sp.	KG5	KP318029	Kos Hot Spring/ Bingol	40	7.5	Metalloprotease	Gul Guven (2007) and Ahmetoglu et al. (2015)
Bacillus sp.	–	–	Alangullu hot spring/Aydin	65	6.0	Esterase	Bakir Ateslier and Metin (2006)
Bacillus subtilis	–	–	Diyarbakir	37	7.0	Amylase	Ozdemir et al. (2011)
Bacillus sp.	ANT-6		Adana	20–55		Amylase	Arikan et al. (2003)
Bacillus thermoamylovorans	O10, O15, O21	KM596795, KM596800, KT364469	NED[2]	56	7.0	Amylase, protease, lipase	Baltaci et al. (2016)
Bacillus thermoamylovorans	A11	KC310462	NED[1]	57±1	6.5–9.0	Amylase, cellulase	Yanmis et al. (2015)
Bacillus thermolactis	A6	KC310457					

Aeribacillus pallidus	O1, O3, O5, O6, O11	KM668038, KM596788, KM596790, KM596791, KM596796	NED[2]	56	7.0	Amylase	Baltaci et al. (2016)
Aeribacillus pallidus	P26	EU935591	Pasinler hot spring/ Erzurum	55	7.5–8.5	Catalase, peroxidase, removal of some textile dyes	Taslimi et al. (2013)
Aeribacillus pallidus	C10	KC333049	Belkisana hot spring/Sirnak	55	9.0	Protease	Yildirim et al. (2017)
Brevibacillus aydinogluensis	PDF25^T	NR_117986	Karakoc hot spring/İzmir	55	7.0	–	Inan et al. (2012)
Brevibacillus sp.	Z1	KC292196	Diyadin hot spring/Agri	59 ± 1	6.5-8.5	Amylase, cellulase, laccase, removal of some textile dyes	Bozoglu et al. (2013) and Yanmis et al. (2015)
Brevibacillus gelatini	PDF4^T	KP899808	Camkoy hot spring/Aydin	40	7.0	Gelatinase	Inan et al. (2016)
Thermomonas hydrothermalis	O4	KM596789	NED[2]	56	7.0	Amylase, lipase	Baltaci et al. (2016)
Thermus anatoliensis	MT1^T	NCCB100425^T	Buharkent geothermal area/Aydin	65	7.5	β-Glucosaminidase; the valine, leucine and cystine aminopeptidases; lipase (C14); α-galactosidase; α-glucosidase; alkaline; and acid phosphatases	Kacagan et al. (2015)

NED: The samples collected from various hot springs and the source is **not exactly defined**

NED[1]: The samples collected from Kirsehir–Terma, Sirnak/Guclu Konak–Belkis Ana, Agri–Diyadin, Kayseri–Haciveli, Erzurum–Ilica, Urfa–Karaali, Rize–Ayder, Adiyaman–Kurucay and Ankara–Kizilcahamam

NED[2]: The samples collected from Bademli Bahce hot spring (Bursa), Sulusaray hot spring (Tokat), Sicak Cermik hot spring (Sivas), Bostanci hot spring (Balikesir) and Diyadin hot spring (Agri)

B. pumilus and two strains resembled to *B. licheniformis* (all at a similarity ratio of 99%). However, three isolates were very close to *B. thermoamylovorans* (≥99%), and two belonged to *Anoxybacillus* genus. In addition, one of the isolates belonged to *Thermomonas hydrothermalis* (with 99% similarity). These isolated thermophilic bacteria were also evaluated for their capability to produce enzymes such as protease, lipase, cellulase and amylase (Baltaci et al. 2016).

Inan et al. (2012) isolated two moderately thermophilic, Gram-positive, endospore-forming, rod-shaped, motile bacteria, designated as PDF25^T and PDF30 from water and mud samples of Karakoc hot spring (the water temperature is around 60–70 °C) in Izmir Province. Cells grow at a temperature range of 35–65 °C (optimum of 55 °C) and pH 6.0–0 (optimum pH of 7.0), hydrolysing casein, starch, gelatin and ONPG (o-nitrophenyl-beta-D-galactoside). The strain PDF25^T was identified as *Brevibacillus aydinogluensis* characterised by gene sequence analysis of 16S rRNA. It was demonstrated that both strains were the members of the genus *Brevibacillus*; the strain PDF25^T had a high sequence similarity to *Brevibacillus thermoruber* DSM 7064 T (98.5%). DNA–DNA hybridisation results displayed 58% relatedness between the strain PDF25^T and *B. thermoruber* DSM 7064^T. The G + C content of genomic DNA was 56.09 mol %. Based on phenotypic and genetic characterisation (particularly by the analysis of the sequence of hypervariable (HV) region), the strain PDF25^T distinguished as a novel species of the genus *Brevibacillus*. Furthermore, Inan et al. (2016) isolated two moderately thermophilic, Gram-positive, endospore-forming, motile, rod-shaped bacteria from Camkoy hot spring Aydin Province, Turkey. The strain designated as PDF4^T had a DNA G + C content of 51.7 mol %, and DNA–DNA hybridisation of strain PDF4^T and type strains of the closely related species displayed less than 60% relatedness. For the type strain PDF4^T (=NCCB 100559^T = DSM 100115^T), the species name of *Brevibacillus gelatini* sp. nov. was proposed.

In a recent study, Kacagan et al. (2015) have isolated a Gram-negative, aerobic rods, nonmotile, catalase, urease and oxidase-positive bacterium (strain MT1^T) from Buharkent hot water in Aydin city of Turkey (water temperature and pH were 88 °C and 6.5, respectively). The strain hydrolysed starch and gelatin, as well as possessing a variety of enzyme activities including β-glucosaminidase, valine, leucine, and cysteine aminopeptidases, lipase (C14), α-galactosidase, α-glucosidase, acid and alkaline phosphatases, which need to be exploited for possible uses in biotechnology. The isolated strain was found to grow at temperature range of 45–80 °C (with optimum of 65 °C) and at pH 5.5–10.5 (with optimum of 7.5). The comparison of16S rRNA gene sequence similarity values between strainMT1^T and other *Thermus* species revealed highest similarity of 96.92% to *T. islandicus* PRI 383^T, followed by *T. arciformis* TH92^T (96.48%) and *T. composti* K-39^T (95.73%). The G + C content of strain MT1^T genomic DNA was 69.6 mol %. On the basis of various methodologies using a polyphasic approach, strain MT1^T was suggested as a novel species named as *Thermus anatoliensis*. The type strain is MT1^T (=NCCB 100425 ^{T} = LMG 26880^T).

8.4 Biotechnological Importance of Thermophiles Isolated in Turkey

8.4.1 Possible Applications Related to Enzyme Industry

Thermozymes have been widely used in many industrial applications as they are well known to possess thermal tolerance and stability to harsh industrial processes at very high temperatures (Demirjian et al. 2001). Microbes and their enzymes are used in a wide range of biotechnological applications such as detergent, fine chemicals, pharmaceutical, bioremediation, food, leather, paper and textile industry. The most important enzymes for industry are lipases, carboxylesterases, cellulases, xylanases, pectinases, amylases, galactosidases and proteases. Thermophilic bacilli are the natural source of most thermostable enzymes. As can be seen in Tables 8.1 and 8.3, a number of thermozymes from thermophilic bacilli isolated in Turkey are as follows: those belonging to *Bacillus* species such as amylase (Arikan et al. 2003; Ozdemir et al. 2011; Matpan Bekler et al. 2015b), chitinase (Sandalli et al. 2008), metalloprotease (Matpan Bekler et al. 2015b; Ahmetoglu et al. 2015) and β-galactosidase (Matpan Bekler et al. 2015a) and to the *Anoxybacillus* species such as α-amylase (Matpan Bekler and Guven 2014; Acer et al. 2015, 2016), xylanase (Kacagan et al. 2008; Inan et al. 2013), glucosidase (Cihan et al. 2011a), glucose isomerase (Karaoglu et al. 2013), ribulokinase (Tokgoz et al. 2014), esterase (Colak et al. 2005; Faiz et al. 2007; Ay et al. 2011), lipase (Bakir and Metin 2015 and 2017), aldolase (Ertunga et al. 2007), CTP synthase (Sandalli et al. 2014), β-galactosidase (Matpan Bekler et al. 2017) and protease (Matpan Bekler et al. 2015c) which have been well characterised. There have been also several studies on the novel enzymes of thermophile *Geobacillus* sp. isolated from Turkey (see Table 8.2), including xylanase (Canakci et al. 2012; Cakmak and Saglam Ertunga 2017), arabinofuranosidase (Canakci et al. 2007), α-glucosidase (Cihan et al. 2009, 2011b), and DNA polymerase I (Caglayan and Bilgin 2011, 2012). Kocabiyik and Erdem (2002) also studied on alkaline proteases produced by various thermoacidophilic archaeal and bacterial strains growing optimally around pH 2.0–5.0, which were originally isolated from acidic hot springs in various hydrothermal sites in Turkey. Here, we summarise on various thermostable enzymes and their characterisation in studied thermophiles that are isolated in hot springs or geothermal areas in Turkey (Tables 8.1, 8.2 and 8.3).

It is very clear that *Anoxybacillus* species are most studied microorganism in hot springs of Turkey, in terms of both identification and their thermostable enzymes. For example, Colak et al. (2005) reported on *A. gonensis* G2 secreting an esterase responsible for the degradation of poly-3-hydroxybutyrate (P3HB). The optimum enzyme parameters were pH 7.5 and 60 °C. The enzyme activity was enhanced by Ca^{2+} indicating to be a cofactor which is a characteristic for lipases/esterases. The esterase activity is inhibited by the metal chelating agent ethylenediaminetetraacetic acid (EDTA), supporting its metalloenzyme characteristic. A similar study was carried out by Faiz et al. (2007) on a thermostable esterase in a novel thermophile, *A. gonensis* A4, capable to degrade tributyrin. The extracellular enzyme had a

molecular weight of 62 kDa. The optimum pH and temperature values for the esterase of strain A4 were 5.5 and 60–80 °C, respectively. They also showed that the enzyme esterase had serine residue in active site and –SH groups were found to be essential for enzyme activity. In addition, a gene encoding a thermostable carboxylesterase from *Anoxybacillus* sp., PDF1, was cloned in *Escherichia coli* BL21. The molecular mass of purified recombinant protein was about 26 kDa as determined by SDS–PAGE. The enzyme showed activity under a wide pH (pH 5.0–10.0) and temperature range (25–90 °C) with optimum temperature and pH values of 60 °C and 8.0, respectively. The inhibition tests on carboxylesterase of *Anoxybacillus* sp. PDF1 revealed that it possesses a serine residue in active site and –SH groups in specific sites, required for its activity (Ay et al. 2011). Lipases and esterases are well known to catalyse many reactions such as esterification, interesterification, alcoholysis or acidolysis, used for fat and oil industry, in the synthesis of flavour esters for food industry, for the synthesis of fine chemicals in the pharmaceutical industry.

Bakir and Metin (2015) isolated 201 thermophilic bacteria from a hot spring in Alangullu/Aydin (50 °C). Among these, 22 isolates exhibited lipase activity. However, the strain HBB 134 having a maximum 16S rRNA sequence similarity (99%) with *Anoxybacillus flavithermus* was found to be the best lipase-producing isolate, which was the first report for a lipase production in the genus *Anoxybacillus*. In another study, the authors isolated another thermophilic lipase-producing bacterium, namely, *Anoxybacillus* sp. HBB16, showing 16S rDNA sequence similarity of 96% with *A. flavithermus.* The maximum activity of the alkaline lipase occurred at 55 °C and pH 9.5 (Bakir and Metin 2017). Lipases (triacylglycerol acylhydrolase; EC 3.1.1.3) are biotechnologically important enzymes which catalyse the hydrolysis of mono-, di- and triacylglycerides to glycerol and free fatty acids at an oil–water interface.

Another thermostable enzyme, which has a biotechnological importance, is amylase, used in many other industrial areas, such as in removing food and starch stains in dry cleaning, in the textile, starch and food industry, and in the purification of apple and pear juice, in the detergent and pharmaceutical industries. Matpan Bekler and Guven (2014) carried out a study on a novel α-amylase produced by a newly isolated thermophilic bacterial strain, namely, *Anoxybacillus* KP1 from the Diyadin hot spring (water temperature 50 °C, pH 7.4) in Agri Province, in northeastern Turkey. Maximal activity of the α-amylase was observed at the pH and temperature of 8.0 (pH range at 6.0–10.0) and 60 °C, respectively. The α-amylase production increased in the presence of 2% (w/v) soluble starch, some nitrogen sources and Mn^{2+}. The enzyme was calcium-independent, considerably stable at a range of pH and temperature, which may be advantageous in industrial applications for food processing and traditional brewing, where the temperatures could denature the enzymes after fermentation. Moreover, an extracellular α-amylase production by a novel thermophilic *Anoxybacillus* sp. AH1 from Dargecit hot springs in Turkey was investigated in the presence of many different media containing a variety of carbon and nitrogen sources. It was also found that α-amylase from *Anoxybacillus* sp. AH1 was thermostable and Ca^{2+} dependent (Acer et al. 2015). In a more recent study,

Acer et al. (2016) purified this α-amylase from *Anoxybacillus* sp. AH1 and determined the molecular mass as 85 kDa. The enzyme had the optimum temperature and pH values of 60 °C and 7.0, respectively. The enzyme activity was seen to increase by various detergents, Mg^{2+} and Ca^{2+}, but there was a significant inhibition by metal ion chelators 1,10-phenanthroline and EDTA. In addition, the activity of α-amylase was enhanced by dithiothreitol (DTT) and β-mercaptoethanol, but it was inhibited by p-chloromercuribenzoic acid (PCMB), indicating the presence of one essential cysteine residue at least in the enzyme active site. The strain AH1 α-amylase inhibition by phenylmethylsulfonyl fluoride (PMSF) also indicated the importance of the seryl hydroxyl group in the catalysis of this enzyme.

A thermophilic *Anoxybacillus ayderensis* AB04^T that was isolated from the Ayder hot spring was found to possess a number of glycoside hydrolases (GHs) which are of importance for carbohydrate-related industries. The GHs of *A. ayderensis* AB04^T were compared to those of other sequenced *Anoxybacillus* spp. genomes, where 14 GH enzyme genes encoded in the genome that belong to GH families 1, 10, 13, 31, 32, 51, 52 and 67 were detected. It was predicted that nine GH enzymes were active on α-chain polysaccharides (pullulanase, α-amylase, α-glucosidase, CDase and oligo-1,6-glucosidase), while the other five GH enzymes act specificity on β-linked polysaccharides (i.e., xylan and cellulose). Those uniquely present were endo-1,4-β-xylanase (NCB I locus ID: KIP 21668) and α-glucuronidase (KIP 21917). Despite the GHs, other *A. ayderensis* AB04^T enzyme genes coding for industrially important enzymes were esterase, aldolase and xylose isomerase. Particularly, xylose isomerase (EC 5.3.1.5) catalyses the isomerisation of glucose to fructose and of xylose to xylulose, which is important in the industry of high-fructose corn syrup production. Two esterases (KIP 19922 and KIP 21735) were detected in the strain AB04^T genome, which had 96.0% and 96.3% amino acid sequence similarity with the esterase from *A. gonensis* G2^T and *Anoxybacillus* sp. PDF-1, respectively. Moreover, *A. ayderensis* AB04^T contains two aldolases, KIP 21451 and KIP 21450 (Belduz et al. 2015).

Complete genome sequencing of *Anoxybacillus gonensis* type strain G2^T (=NCCB 100040^T = NCIMB 13,933^T) isolated previously from Gonen hot springs showed that this strain consisted various carbohydrases, such as pullulanase (AKS39285) and α-amylase (NCBI locus ID: AKS37565), which are valuable for starch hydrolysis in industry, cyclodextrinase (AKS37561) used in cyclodextrin-related research and also xylose isomerase (AKS39170) and β-xylosidase (AKS39172) which are good candidates for second-generation biofuel production. They also reported on some other novel enzymes of *A. gonensis* G2^T and their potential use in biotechnology, such as β-galactosidase (AKS39183), α-galactosidase (AKS39187), oligo-1,6-glucosidase (AKS37459) and α-glucosidase (AKS37566). This strain was found to produce many other well-studied enzymes with biotechnological importance, including fructose-1,6-bisphosphate aldolase and carboxylesterase (Lim et al. 2015). Moreover, in the thermophile *A. gonensis* G2^T, a new glucose isomerase (GI) was described by Karaoglu et al. (2013), which is particularly suitable for the production of high-fructose corn syrup in the food industry. The gene encoding this enzyme was cloned and expressed in *E. coli.* The purified

recombinant enzyme, with a molecular weight of approximately 50 kD determined by SDS–PAGE and MALDI–TOF analysis, had an optimal activity at 85 °C and pH 6.5. In a study carried out by Sandalli et al. (2014), a novel CTP synthase gene of *A. gonensis* G2 was cloned, expressed and characterised. The thermophilic cytidine-5′-triphosphate synthase (EC 6.4.3.2) gene (pyrG) was 1590 bp long encoding a protein with 530 amino acids, possessing a molecular weight of 59.5 kDa. The CTP synthase amino acid sequence showed a similarity of 90%–94% with *Bacillus* sp.

A ribulokinase from *Anoxybacillus kestanbolensis* AC26Sari isolated from the hot spring mud (Camkoy in Canakkale province, Turkey) was studied, cloned and expressed in *E. coli* BL21 (Tokgoz et al. 2014). The ribulokinase of the strain AC26Sari was found to have 99% DNA and 99% amino acid identity with ribulokinase of *A. flavithermus* WK1, while 90% DNA and 96% amino acid identity with *Geobacillus thermodenitrificans* NG80–2 ribulokinase. The purified enzyme had a molecular mass about 61 kD, as determined by SDS–PAGE, and was found to be active at a wide temperature (50–75 °C) and pH (pH 5.0–10.0) range, with optimum temperature of 60 °C and an optimum pH of 9.0. The activity of purified enzyme was strongly inhibited by Zn^{2+} but enhanced by Mg^{2+}, though the ribulokinase from *A. kestanbolensis* AC26Sari did not require any other metallic cations for its activity. This was the first report to characterise a thermophilic ribulokinase of thermophilic bacteria. L-ribulokinase is unusual among kinases because it is known to phosphorylate all four 2- ketopentoses (L- or D-xylulose and L- or D-ribulose).

Xylanases are well known to be important in biotechnology increasing the nutritional quality of animal feed and in the textile fibre recovery and used for industrial wastes in pulp and paper industry, as well as in the clarification of fruit juices, wine, etc. A thermophilic, xylanolytic bacterium isolated from the Diyadin hot springs was identified as *Anoxybacillus pushchinoensis* strain A8 by sequence similarity of 16S rRNA gene and DNA–DNA hybridisation studies. The extracellular xylanase had a molecular mass of approximately 83 kDa. The maximal activity obtained for the enzyme was pH 6.5 (stable over a broad pH range of 6.5–11 for 24 h) and 55 °C (stable at temperature between 50 and 60 °C up to 24 h). The enzyme was found to be an exo-acting xylanase (Kacagan et al. 2008). In another study, Inan et al. 2013 found that *Anoxybacillus kaynarcensis* produced xylanase activity, and the zymogram analysis of SDS–PAGE revealed apparent molecular weights between 100 and 150 kDA, with the optimum temperature and pH values of 65 °C and 7.0–9.0, respectively.

Proteases, particularly thermostable ones, have been used for a long time for many industrial applications. In search of proteases, Matpan Bekler et al. (2015c) studied a novel extracellular alkaline protease (EC 3.4.21–24, 99) in thermophilic *Anoxybacillus* sp. KP1 strain. The purified enzyme had a molecular weight of 106 kDa using SDS–PAGE, which was stable at pH 9.0 and at 50–60 °C for 1 h. Some chemicals such as Triton X-100, Tween 80, Ca^{2+} and Cu^{2+} increased the activity of the enzyme, while EDTA and PMSF inhibited proteolytic activity, suggesting that the enzyme was a serine alkaline protease. They also stated that the detergent

stability (residual activity between 73% and 82%) was an important feature for their industrial applications, such as detergent industry.

The genus *Geobacillus* has also drawn attention due to their potential use in biotechnology. Canakci et al. (2007) investigated on thermophilic xylanase and arabinofuranosidase activities in the isolated 16 Gram-positive bacilli which belonged to the genus *Geobacillus* from Dikili–Bergama Kaynarca hot spring (Izmir Province) and Camkoy Camur, Omerbeyli and Alangullu hot springs in Aydin Province in Turkey. They reported that seven of the isolates had both arabinofuranosidase and xylanase activities, while four of them had only xylanase and the other five isolates had none of both activities. The xylanase of isolates 3.3, 7.1 and 9.1 had the highest optimum temperature of 80 °C, while the isolates AO4, AO17, 7.2, 9.1 and 9.2 had the highest optimum pH of 8. The optimum temperature for arabinofuranosidase activity for isolates 7.2, AO4, AC15 and 12 was 75 °C, whereas only isolate AC15 had the lowest pH of 5.5.

A xylanase-encoding gene from *Geobacillus* sp. 7.1, isolated from the hot spring of Dikili–Bergama Kaynarca, was cloned and sequenced, followed by overexpression in *E. coli* and purification. Extracellular xylanase having a molecular weight of 47 kDa had the optimum pH and temperature values of 8.0 and 75 °C, respectively. The xylanase had the most sequence similarity (93%) with the enzyme from *G. thermodenitrificans* NG80–2. It was found that the enzyme carried a catalytic domain which belonged to the glycoside hydrolase family 10 (GH10), exhibiting an excellent pH stability. The enzyme did not have cellulase activity, whereas degraded xylan in an endo-fashion (Canakci et al. 2012). In addition, Cakmak and Saglam Ertunga (2017) have recently studied on cloning, expression, immobilisation and characterisation of an endo-xylanase and its industrial applications in *Geobacillus* sp. TF16 collected from the Germencik Omerbeyli hot spring in Aydin. The molecular weight of the recombinant enzyme was found to be a single band of 39.8 kDa on SDS–PAGE. The immobilised enzyme compared to free enzyme showed an increase in optimum temperature from 55 to 65 °C. The optimum temperature for the free enzyme was pH 8.5, whereas immobilised enzyme displayed a higher activity in the pH range 6.0–8.5. The endo-xylanase was shown to have importance for use in biotechnology as it was capable of releasing the reducing sugar from juice and poultry feed and oven spring in bakery.

A study was performed on the purification and characterisation of novel DNA polymerases of *Geobacillus kaue* strain NB isolated from Gonen and Hisaralan hot springs in Turkey. It was shown that the optimum values for the enzymatic activity of *G. kaue* polI was 70 °C and pH 7.5–8.5. In addition, polyamines stimulated the polymerisation activity of the enzyme. Three-dimensional structure of polI showed that all functionally important regions were conserved in the polymerase active site computed using homology modelling (Caglayan and Bilgin 2011).

The thermophilic chitinases that degrade chitin, the most abundant renewable natural resource after cellulose, have a wide range of biotechnological applications. A chitinase gene (chiB65) in *Bacillus licheniformis* A1 obtained from Diyadin hot spring was cloned and expressed in *E. coli* and then sequenced. The purified recombinant protein was analysed on SDS–PAGE using the fluorogenic substrate

4-methylumbelliferyl β-D-N,N′-diacetylchitobioside, having a molecular weight of approximately 71 kD. The optimum values for the enzyme were pH 6.0 and temperature of 65 °C, though it was stable at a pH range of 5.0–9.0 for 4 h at 65 °C and 24 h at room temperature (Sandalli et al. 2008).

Thermophilic amylases are well known to be used for hydrolysis of starch to produce glucose and related chemicals in industry. From this point of view, an alkaline, thermostable α-amylase-producing *Bacillus* sp. ANT-6 was identified by Arikan et al. (2003). The enzyme had an optimum activity at 80 °C and pH 10.5. The relative molecular mass of the enzyme was found as 94.5 kDa. A *Bacillus subtilis* strain isolated from soil samples in Diyarbakir, Turkey, was also studied for its thermostable α-amylase. The effects of many parameters such as incubation time, different culture media, carbon and nitrogen sources and various starches, flours, detergents and other chemicals on the production of α-amylase were studied. The purified enzyme was found to be Ca-dependent, having the optimum pH and temperature of 6.0 and 60 °C, respectively (Ozdemir et al. 2011).

Ahmetoglu et al. (2015) studied on a novel extracellular protease produced by *Bacillus* sp. KG5 isolated from Kos hot spring (Bingol, Turkey). The molecular weight of purified enzyme was approximately 48 kDa by both native and SDS–PAGE and was not a serine–protease as PMSF did not have an inhibitory effect on protease activity. The enzyme showed maximum activity at pH of 7.0–7.5. It was also determined that the protease was thermostable, particularly fully stable in the Ca^{2+} presence at 50 °C even after 120 min. It is clear that thermostability of proteases is a critical feature required for industrial applications such as leather processing and detergent. Proteases are also used in many applications such as bioremediation, biotransformation and biosynthesis, brewing, food, meat, dairy industries and diagnostics. In a newly isolated thermophilic *Bacillus licheniformis* DV3, extracellular thermostable α-amylase and protease were studied. The optimum temperature and pH values for both extracellular enzymes were 70 °C and 7.0 for the α-amylase, respectively, while it was 10.0 and 50 °C for the protease, respectively. The α-amylase activity was enhanced in the presence of Mn^{2+}, inhibition was obtained in the presence of Ca^{2+} indicating to be a member of calcium–independent amylases. The protease activity increased in the presence of Ca^{2+} and Zn^{2+}, whereas the activity was decreased by EDTA and PMSF, indicating that the enzyme was a metallo- and serine protease (Matpan Bekler et al. 2015b).

A thermostable β-galactosidase from a thermo- and alkalitolerant KG9 strain belonging to *Bacillus licheniformis* isolated from Taslidere hot spring in Batman (Turkey) was cloned, expressed in *E. coli* and characterised. Due to genomic sequence similarity of *B. licheniformis* strain KG9 to that of *B. licheniformis* strain DSM 13 (99.9% identity), PCR primers based on four putative β-galactosidase genes in the genome of strain DSM 13 were employed for the isolation of the corresponding β-galactosidase genes from KG9 strain. The molecular masses of β-galactosidases I, II, III and IV were calculated as 30, 79, 74 and 79 kDa, respectively, using sequencing data. Similarly, the number of identified β-galactosidase genes in strain KG9 was four, and three genes were expressed in *E. coli* as intracellular and active. Among these three, the authors purified and characterised the

recombinant β-galactosidase III, having the optimal pH and temperature of 6.0 and 60 °C, respectively. The purified enzyme analysed on SDS–PAGE displayed one single band with a molecular weight of ~75 kDa (Matpan Bekler et al. 2015a). It has been also claimed that the characteristic such as thermostability makes this recombinant β-galactosidase favourable in the application of β-galactosidase in dairy and food processes involving hydrolysis of lactose in order to enhance the digestibility of milk or to improve the functional characteristics of milk products, etc.

8.4.2 Applications Related to Environmental Biotechnology

The use of thermophiles and their bioproducts in environmental biotechnology is well known such as biohydrogen production, bioconversion of lignocellulose to hydrogen, conversion of glycerol to lactate, conversion of D-xylose into ethanol, biodegradation of dyes or petroleum hydrocarbons, recovery of heavy metals, etc. The thermophiles and their products are resistant to harsh conditions in industrial applications by supplementing or replacing traditional chemical processes (Mehta et al. 2016).

The H_2-producing bacteria, closely affiliated to genus *Thermoanaerobacterium* determined by PCR–DGGE profiling, were isolated from hot spring of Hisaralan in Balikesir Province, Turkey. H_2 bioproduction was accompanied by production of acetate, butyrate, ethanol and lactate. It was found that H_2 production was maximum at the temperature range from 49.6 to 54.8 °C (Karadag et al. 2016).

The dyes are commonly used in different industrial fields such as textile, food, cosmetics and paper; on the other hand, they cause health and environmental problems. The enzyme called laccase (oxidoreductase) has ability to oxidise the compounds associated with both phenolic and nonphenolic lignin and to deoxidise the pollutants resistant to biodegradation, for example, used in the removal of textile dyes, phenols and detoxification of wastes. In a study carried out by Yanmis et al. (2016), an extracellular laccase from *Anoxybacillus gonensis* P39 (Gen Bank No:FJ808725) isolated from Ilica hot spring, Erzurum Province, was purified with a molecular weight of 40 kDa on SDS–PAGE and with optimum pH and temperature values of 5.0 and 60 °C. Bozoglu et al. (2013) also studied on the purification and characterisation of a laccase (with molecular mass 93 and 110 kDa) and its possible use in removal of textile dyes, from a new thermophilic strain of *Brevibacillus* sp. (Z1) isolated from Diyadin hot springs in Agri Province of Turkey. The evaluation of laccase in both studies for possible use in bioremediation process of some textile dyes showed that the laccase reduced the amount of several dyes such as the Reactive Black 5, Fuchsine, Allura Red and Acid Red 37 in wastewater.

Lim et al. (2015) found that the *Anoxybacillus gonensis* $G2^T$ consisted arsenate reductase (AKS38388) and three mercury (AKS37713, AKS38377, AKS38379) genes in its genome, showing that the $G2^T$ strain may be used in heavy metal bioremediation. The analysis of *A. ayderensis* $AB04^T$ genome showed the presence of at least six heavy metal resistance genes, four of which were mercury resistance (mer)

operons (KIP 20706 and KIP 20408) and mercuric reductases, catalysing the reduction of Hg^{2+} to Hg 0 (KIP 19952 and KIP 20409). Other two genes were arsenate reductase (KIP 20402) and arsenic efflux pump protein (KIP 20401). Beris et al. (2011) also reported on an aluminium tolerance gene (*G2alt*) and the effects of environmental conditions on its biological functioning in the thermophilic $G2^T$ strain. The *G2alt* gene was 666 bp long and encoded a protein of 221 amino acids. The amino acid sequence of the protein with ATPase activity was highly similar to proteins which are responsible for aluminium resistance.

There has been an emphasis given to the utilisation of microorganisms, so as thermophiles for their great metal ion absorption ability from aqueous solutions. Duran et al. (2009) used *A. gonensis* which was immobilised on Diaion HP-2MG as a new biosorption system for the enrichment of various metals prior to the atomic absorption spectrometric analysis. More recently, a thermophilic haloalkalitolerant bacterial strain named KG9 was newly isolated and identified as a close member of *Bacillus licheniformis* which was also evaluated for possible use in environmental technology by Alkan et al. (2015) as a new biosorbent for preconcentrating Cd(II), Ni(II) and Cu(II) prior to flame atomic absorption spectrometric (FAAS) analysis. The strain (KG9) immobilised on Amberlite XAD-4 was used for the measurement of toxic metal ions in real samples such as the Tigris river and drinking water and in mushrooms. The optimum parameters such as eluent type and volume, amount of adsorbent, pH, sample solution volume, sample solution flow rate and matrix interference effect on the metal ion retention were investigated for the analyte quantitative recovery.

8.5 Hypersaline Environments of Turkey

Turkey, especially Central Anatolia, is rich for hypersaline environments. Tuz Lake which is the largest salt lake in central Turkey occupies a depression in the dry central plateau of Turkey, located in 105 km northeast of Konya city and 120 km south of Ankara. The lake stays shallow (1–2 m) and has a total surface area of 1665 km^2, with a length of 90 km and a width of 35 km within a closed basin. The water salt concentration reaches up to 33%. In summer, when the lake dries out, a 30-cm layer of salt forms due to the evaporation. The lake and the salterns provide a main source of solar salt: 73% of the salt consumption of Turkey, meaning that the lake produces more than 200 million tons of salt (Birbir and Sesal 2003; Mutlu et al. 2008). Moreover, Camalti Saltern is the biggest artificial marine solar saltern in Turkey. It is a multipond system consisting of 182 ponds covering 58 km^2 and located about 38°35′N and 26°57′E on the east cost of the Aegean Sea. Sea salt extraction has been carried out in the area since 1863 (Mutlu and Guven 2015).

8.6 Halophilic Microorganisms Isolated from Extreme Environments of Turkey and Their Possible Use in Biotechnology

8.6.1 Halophilic Archaea and Bacteria from Hypersaline Environments of Turkey

A distinct class of extremophiles is halophiles which means that salt is required for them to survive. Halophilic microorganisms are found in various hypersaline environments including crystalliser ponds, saline sand and soils, marine environments, solar lakes and hypersaline lakes. Microorganisms adapted to life at very high salt concentrations are widely spread, both within the archaeal and the bacterial domain which are well known to comprise a well-defined, aerobic or facultatively anaerobic microorganisms (Ozcan et al. 2007; Mutlu and Guven 2015). There have been several studies recently on extremely halophilic communities in various hypersaline environments such as salterns, salt lakes or salt mines in Turkey, for the aim of identification and/or possible biotechnological uses (Table 8.4).

Salt lakes and the solar salt contain huge numbers of prokaryotes, mainly extremely halophilic *Archaea* of the family *Halobacteriaceae* (Birbir et al. 2007). Typical characteristics of the family *Halobacteriaceae* members are having different shades of red as colony colour, various morphological types from rods, cocci, to extremely pleomorphic, and growing at 25% NaCl concentration (Grant et al. 2001). Moreover, all isolates are known to comprise ether-bound membrane lipids as well as being resistant to antibiotics that target the bacterial peptidoglycan (Ozcan et al. 2007). Both metabolic diversity and biotechnological potential have been found in halophilic and halotolerant microorganisms (Tatar et al. 2016).

Birbir and Sesal (2003) studied on extremely halophilic microorganism communities in Sereflikochisar Salt Lake in central Turkey. A research on microbial diversity was carried out in this area due to being a main source of solar salt for food and hide and also due to the economic importance. In total, 82 extremely halophilic aerobic strains from six salt and three brine samples were detected, indicating a diverse bacterial community, 32 of which were randomly selected strains. Most cells of the strains stained Gram-negative and motile. Optimum growth was observed at 40 °C, at a pH of 7.5 and in the presence of 25% (w/v) NaCl. The results of morphological, biochemical and physiological characteristics of the isolates and antibiotic sensitivities were used to distinguish *Archaebacteria* and *Eubacteria* in the lake. It was also demonstrated that the lake accommodated a fairly wide diversity of halophilic species producing industrial enzymes such as cellulases, β-galactosidases, lipases and gelatinases.

Extremely halophilic archaea are well known to survive in the hypersaline conditions such as salt mines or salt lakes. In a hypersaline environment, namely, the Ayvalik saltern, seven extremely halophilic archaea were isolated by Elevi et al. (2004). The characterisation of halophilic strains was based on the conventional methods, including polar lipid composition, exoenzyme production, protein profiles, plasmid size and number. The Ayvalik saltern is also a very important resource

in the country, and the produced salt is widely used in a variety of industrial processes such as food preservation and curing of hides (cheese, pickles, tomato, paste, fish, etc.), as well as in leather industries in Turkey. It also provides a good habitat for halophilic microorganisms. All studied isolates needed at least 15% (w/v) NaCl concentration in the medium to grow. The optimum growth for seven red halophilic *Archaea* strains were salt concentrations ranging 20–25% (w/v) NaCl at 39 °C. The isolates characterised belonged to the archaeal family *Halobacteriaceae*. The red colour (based on α-bacterioruberin derivatives) observed due to the extremely halophilic characteristic of the cultures was evidence for the presence of *Archaea* species. Due to the lipid compositions, triglycosyl diether as glycolipid of four isolates (strains R1–R4) assigned them to the genus *Haloarcula*, while strains R5–R7 containing sulphated diglycosyl diether instead resembled to *Halorubrum saccharovorum*.

A study was also conducted on the microbial diversity in the hypersaline Tuz Lake and its salterns, Kaldirim and Kayacik, located in Central Anatolia, Turkey. This study presented the results on diversity of extremely halophilic *Archaea*. Twenty-seven different strains belonged to the family *Halobacteriaceae*, which are known to be aerobic and possess red or pink pigments, and were characterised based on colony pigmentation, phenotypic characteristics, polar lipid compositions and antibiotic sensitivities. Moreover, gene sequence analysis of 16S rRNA of the isolates was performed, and the phylogenetic analysis revealed that the isolated strains are mostly assigned to the genera *Halorubrum*, *Halobacterium* and *Haloarcula*. In particular, the most dominant genus in Lake samples was *Haloarcula*, while *Halorubrum* members were detected in Tuz Lake and the saltern samples of Kaldirim, and the species of *Halobacterium* were obtained from Tuz Lake and the Kayacik saltern. All archaeal strains possessed hydrolytic enzymes (cellulases, amylases, proteases and others), used in food, detergent and leather industries (Birbir et al. 2007).

Ozcan et al. (2007) reported on the diversity of archaeal strains isolated from water and soil samples of six hypersaline locations in the provinces of Ankara (salt lake, 45.667% salinity), Denizli (Aci Lake, 0.265% salinity), Konya (Bolluk Lake, 48.452% salinity), Kayseri (Tuzla Lake), Kirsehir (Seyfe Lake) and Burdur (Salda Lake, 1.114% salinity). By analyses of morphological and biochemical properties, sensitivity to different antibiotics, plasmids and total lipid composition as well as comparisons of 16S rRNA gene sequences (1388 bp), thirty-three strains were characterised, which all belonged to the family *Halobacteriaceae*. All isolates were found to be Gram-negative, catalase- and oxidase-positive and possessing pink to red colony colour. By phylogenetic analyses, these isolates were clustered into nine genera, namely, *Halomicrobium* (one isolate), *Halalkalicoccus* (one isolate), *Haloterrigena* (three isolates), *Haloferax* (three isolates), *Natrialba* (four isolates), *Natronococcus* (four isolates), *Haloarcula* (four isolates), *Natrinema* (five isolates) and *Halorubrum* (eight isolates).

The prokaryotic diversity in a hypersaline Tuz Lake, Turkey, was also demonstrated by Mutlu et al. (2008). The authors studied microbiota in this lake by using the methodology of FISH, denaturing gradient gel electrophoresis of PCR-amplified

Table 8.4 Isolation, identification and possible biotechnological uses of halophilic microorganisms from various hypersaline environments in Turkey

Microorganisms	Strain code	GenBank accession number	Source	Growth temperature (°C)	Growth pH	Biotechnological applications and products	Reference
Halobacterium sp.	3KYS1, 3TL6, 2KYS1, 5TL6, 3TL4, 2TL9	DQ352855, DQ352856, DQ352857, DQ352858, DQ352859, DQ352860	Kaldirim and Kayacik salt lake	40	7.5	Amylases, cellulases, proteases	Birbir et al. (2007)
Halobacterium sp.	1SL3, 2SL3, 3SL6, 6SL6, 2SL8, 3SL8, 3SL9	–	Sereflikochisar Salt Lake	40	7.5	Cellulases, gelatinases, lipases and β-galactosidases	Birbir and Sesal (2003)
Halobacterium salinarum	1TK2, 1TK3	–	Tuzkoy salt mine	40	6.5–7.5	Amylase, lipase, β-galactosidase, cellulase	Birbir et al. (2004)
Haloarcula vallismortis	3TK1, 3TK2, 3TK3, 2TK2, 2TK3, 4TK1, 4TK3					Amylase, lipase, cellulase	
Natrinema	2TK1, 5TK1					Amylase, lipase	
Halorubrum sodomense	1TK1					Amylase, lipase, DNase, cellulase	

(continued)

Table 8.4 (continued)

Microorganisms	Strain code	GenBank accession number	Source	Growth temperature (°C)	Growth pH	Biotechnological applications and products	Reference
Haloquadratum and *Salinibacter*	1B-8B, 4A-17A	EF459702, EF459730	Tuz Lake	37	–	–	Mutlu et al. (2008)
Halobacillus	EM7, EM14, EM33	KF499274, KF499281, KF499299	Salt-affected soil of Erzurum	30	7.0–11.0	Amylase	Orhan and Gulluce (2015)
Halobacteriaceae	R1–R7		Ayvalik saltern	39	7.2	Amylase, gelatinase	Elevi et al. (2004)
Halobacillus sp.	C-22	HM037269	Camalti Saltern/ Izmir	37	4.5	Decolourisation of textile dyes	Demirci et al. (2011)
Haloferax sp.	C24, C27	JX067385, JX067386	Camalti Saltern/ Izmir	37	6.5–7.5	PAH-degrading enzymes	Erdogmus et al. (2013)
Halobacterium sp.	C37	JX067388					
Halorubrum sp.	C41, C43, C46, C50, C51	JX067389, JX067390, JX067391, JX067392, JX067393					
Halalkalicoccus	C15	DQ373058	Salda Lake/ Burdur	37	7.0	Amylase	Ozcan et al. (2007, 2009)
Haloferax	A440,	DQ309084,	Salt Lake/Ankara Seyfe Lake/ Kirsehir			Amylase, gelatinase	
	A317,	DQ309085,					
	D107	DQ373057					

Halorubrum	F23A,	DQ309087,	Bolluk			Gelatinase	
	C35,	DQ373061,	Lake/Konya				
	F42A,	DQ309086,	Salda Lake/ Burdur				
	A29,	DQ309088,	Salt Lake/Ankara				
	B36,	DQ373060,	Aci Lake/Denizli				
	A87B,	DQ309089,	Seyfe				
	D10,	DQ373059,	Lake/Kirsehir				
	F100	DQ309090					
Haloarcula	A191	DQ309091	Salt Lake/Ankara			Amylase, gelatinase, lipase, esterase	
Halomicrobium	A337,	DQ309092,	Salt Lake/Ankara Aci Lake/Denizli				
	A283,	DQ309093,					
	B44A,	DQ373062,					
	A43	DQ309094					
Halomonas smyrnensis	AAD6^T	DQ131909	Camalti/Izmir	37	5.5–8.5	Amylase, exopolysaccharide, levan production	Poli et al. (2009), Sam et al. (2011), Sogutcu et al. (2012), Poli et al. (2013), Ates et al. (2013) and Calimlioglu and Arga (2016)
Halomonas sp.	AAD12	GU397429	Camalti/Izmir	37	7.0	Hydroxyectoine production, Amylase	Uzyol et al. (2012) and Ozturk et al. (2015)

(continued)

Table 8.4 (continued)

Microorganisms	Strain code	GenBank accession number	Source	Growth temperature (°C)	Growth pH	Biotechnological applications and products	Reference
Halomonas sp.	LM1C	KT588 664	Pamukkale/ Denizli	21.6	6.9	Lipase, esterase	Gutiérrez Arnillas et al. (2016)
Halomonas halophila	C13,	HM0372678,	Camalti marine solar saltern/Izmir	37	6.5–7.5	–	Mutlu and Guven (2011, 2015)
	C18,	KF863791,					
	C20	KF863792					
Halobacillus sp.	C12,	KF863788,					
	C17,	KF863790,					
	C25	KF863793,					
Virgibacillus marismortui	C15	KF863789					
Halolamina sp.	YKT1	KU051660	Yozgat salt mine	37	7.5	–	Kurt Kizildogan et al. (2017)
Streptomonospora tuzyakensis	BN506^T	KCTC 29210^T	Tuz (Salt) Lake, Konya	37	6.0–12.0	–	Tatar et al. (2016)

16S rRNA genes, and by comparisons of the clone library 16S rRNA gene sequences. Interestingly, the number of *Archaea* members in the community was three times more than those of *Bacteria* detected by FISH. The archaeal members were dominantly clustered into the square *Haloarchaea* of the Walsby group, while bacterial members dominantly grouped into *Bacteroidetes*, such as *Salinibacter ruber*-related phylotypes. It is well known that *Bacteroidetes* species are widespread in various hypersaline environments. The comparison between 16S rRNA sequences from the Tuz Lake bacterial strains and those from other hypersaline environments showed a 'halophilic branch' within the *Bacteroidetes* phylum that clustered together.

Moreover, a natural reserve in Sasali, Izmir, in the Aegean Region was studied for the isolation and identification of halophiles from several pond soil samples with salt contents in the range 30–50% and pH in the range of 6.5–7.5. The isolated strains designated as AAD6^T, AAD4, AAD17 and AAD21 were Gram-negative, exopolysaccharide-producing and moderately halophilic bacteria which grew at an optimum of 10% (w/v) NaCl. The G + C compositions of the genomic DNAs of AAD21 AAD17, AAD4 and AAD6^T were 62.6, 62.8, 63.3 and 63.0 mol %, respectively. Sequence comparisons of 16S rRNA gene between the strain AAD6^T and most related species indicated that the strain AAD6^T was close to *Halomonas salina* F8-11^T (99.4% similarity) and *Halomonas halophila* CCM 3662^T (99.4%), and the mean values of DNA–DNA hybridisation between the representative strain AAD6^T and the most related species mentioned above were calculated as 40.8 and 39.6%, respectively. On the basis of the phenotypic, phylogenetic and genomic properties presented above, the strain AAD6^T represents a novel species of the genus *Halomonas* and thus named *Halomonas smyrnensis* (=DSM 21644^T = JCM 15723^T). *H. smyrnensis* was rod-shaped and formed circular and slightly irregular colonies with cream-yellowish colour and was also different from all closely related species of the genus *Halomonas* in terms of hydrolysing starch and casein. This novel species also produced a higher yield of exopolysaccharide named levan which was previously described as repeating unit comprised of beta (2,6)-D-fructofuranosyl residues (Poli et al. 2009, 2013). Moreover, whole genome sequencing of *H. smyrnensis* AAD6^T was succeeded by Sogutcu et al. 2012.

Orhan and Gulluce (2015) have recently mentioned about the importance of halophilic and halotolerant microorganisms in salt-affected soils, which may possess basic enzyme activities that can enhance nutrient cycling and fertility in soil. This study was carried out in salt-affected soil of Erzurum Province in the East Anatolian Region of Turkey. Forty-five bacterial strains were isolated and characterised by phenotypic and phylogenetic techniques. The strains isolated from salt-affected soils belonged to 16 different genera, as follows: *Bacillus* (19 strains), *Staphylococcus* (3 strains), *Halobacillus* (4 strains), *Zhihengliuella* (2 strains), *Oceanobacillus* (2 strains), *Halomonas* (1 strain), *Nesterenkonia* (2 strains), *Promicromonospora* (2 strains), *Jeotgalibacillus* (2 strains), *Planococcus* (2 strains), *Virgibacillus* (1 strain), *Terribacillus* (1 strain), *Thalassobacillus* (1 strain), *Marinibacillus* (1 strain), *Gracilibacillus* (1 strain) and *Microbacterium* (1 strain). They claimed that the characterised strains in salt-affected soils had high salt

tolerance and significant enzyme activities which may be used for improvement of agricultural soils.

Another recent study to identify the bacterial diversity of Camalti marine solar saltern has been carried out by Mutlu and Guven 2015. The total salt concentrations and the pH values of samples collected from this area were measured between 6% and 32% and pH 6.5 and 7.5, respectively. The bacterial communities of Camalti Saltern were characterised by molecular techniques that included the analysis of PCR-amplified fragments of 16S rRNA gene by the denaturing gradient gel electrophoresis. They identified a total of 42 isolates at the genus/species level, and 17 of them belonged to the *Bacteria* domain. All of bacterial strains were phylogenetically related to *Halomona*s, *Halobacillus* and *Virgibacillus* genus. 16S rRNA sequence analysis of the clones by ARDRA method showed that most (85%) of the bacterial clones were the members of *Salinibacter* genus within the *Bacteroidetes*.

A novel halophilic actinobacterium was isolated from Tuz Lake soil sample in Konya by Tatar et al. (2016). The isolate designated as BN506^T was associated with members of the genus *Streptomonospora* based on morphological and chemotaxonomic properties. Moreover, analysis of 16S rRNA gene sequence and DNA–DNA relatedness showed that strain BN506^T was a member of a new species of the *Streptomonospora* genus, named as *Streptomonospora tuzyakensis* (= DSM 45930^T = KCTC 29210^T). The 16S rRNA gene sequence similarities between strain BN506^T and related species showed close relation to *S. halophila* YIM 91355^T (98.1%) and *S. arabica* S186^T (97.9%), with also DNA relatedness values of 41.0 ± 3.5% and 25.2 ± 3.6%, respectively. The genomic DNA G + C content was detected as 71.1 mol %. The isolate was aerobic, Gram-positive, nonmotile actinomycete. The aerial mycelium of the species was white and found to grow at 4–20% NaCl (w/v) and between temperatures of 28 and 37 °C (optimally 37 °C in 10% (w/v) NaCl) and between a pH range of 6.0–12.0.

A global transcriptome analysis has been recently conducted by Kurt Kizildogan et al. (2017) in an extremely halophilic archaeon, *Halolamina* sp. YKT1, isolated from a sample in Yozgat salt mine, in order to explore the molecular mechanisms leading to the high salt tolerance. It was found that the salt tolerance of the YKT1 strain involves the up-regulation of genes related with osmoprotectant solutes, membrane transporters, oxidative stress proteins, CRISPR–Cas systems and iron metabolism. This comprehensive transcriptome analysis however showed that the genes that encode the proteins involved in translation, transcription, DNA replication and DNA repair were downregulated.

8.6.2 Biotechnological Applications of Halophilic Microorganisms Isolated in Turkey

There are promising studies on halophilic bacteria and archaea, as they have ability for producing biochemicals, which possess a significant potential use in industrial and environmental technology (Oren 2010). Furthermore, halophilic bacteria have ability to produce biopolymers that are used in industrial and medical technology

(Table 8.4). For instance, levan is an extracellular biopolymer produced by a moderately halophilic bacterium *Halomonas* species (Poli et al. 2009, 2013; Ates et al. 2013). Moreover, exopolysaccharide-producing halophile, namely, *Halomonas* sp. AAD6 (DQ131909), was isolated from Camalti Saltern Area in Turkey. The strain cultivated on agro-industrial waste produced exopolysaccharides which had a potential use as an alternative and easily biodegradable polyelectrolytes, compared to synthetic ones that are commonly in use and contain toxic and carcinogenic monomers (Sam et al. 2011). The activated sludge culture supplemented with a salt tolerant, *Halobacter halobium*, was utilised for saline wastewater treatment in order to alleviate salt inactivation effects in a biodisc contactor (Kargi and Dincer 1998).

A moderate halophile identified as *Halomonas* sp. AAD12 from salt sediments in Camalti Saltern Area in Turkey was isolated by Ozturk et al. (2015), pointing out that it was a promising candidate as a hydroxyectoine producer. *Halomonas* sp. AAD12 was found to adapt stress conditions by changing its osmolyte accumulation ratio and the fluidity of membrane to prevent the effects of stress. A number of moderate halophiles are known to change the accumulated concentrations of the osmoprotectants ectoine, hydroxyectoine and proline to protect its cytoplasm during stress exposure, such as oxygen limitation, temperature and salinity. These molecules are desired for a variety of uses in biotechnology, for protection of enzymes against different stress factors such as freeze-thawing, freeze-drying and heating and also as a protection for the healthy cell desiccation during chemotherapy and for medical use as a molecular chaperon for Alzheimer's disease, as well as for preservation of cardiac death donors (DCD) livers.

An application field of halophilic bacteria has been the use for biodegradation of dyes. For example, a report was carried out about the use of halophilic bacterium isolated from water and soil samples of a solar sea saltern (Camalti, Turkey) in environmental technology, especially in textile industry for the decolourisation of some of azo–metal complex dyes. Among these, only one bacterium identified by 16S rRNA gene sequence analysis as *Halobacillus* sp. C-22 (99% sequence similarity) was determined as resistant against two dyes, which are Lanaset Brown B and Lanaset Navy R. Following exposure to Lanaset Brown B, the bacterium decolourised the dye at a high absorbance ratio (96.12%) after 78th h, while Lanaset Navy R was significantly decolourised in 10 min by 46.67% and 60.66% at the third hour (Demirci et al. 2011).

Another application field was the use of archaeal isolates in environmental technology for degradation of polyaromatic hydrocarbons (PAHs). Erdogmus et al. (2013) isolated some archaeal strains from the Camalti Saltern (Turkey) and identified them by 16S rRNA gene sequences as *Halobacterium salinarum*, *Halobacterium piscisalsi*, *Halorubrum ezzemoulense*, *Haloarcula hispanica*, *Haloarcula* sp., *Haloferax* sp. and *Halorubrum* sp., which were found to degrade PAHs (namely, naphthalene, p-hydroxybenzoic acid, pyrene and phenanthrene) to use for the carbon and energy sources. Recently, halophilic microorganisms have been also used for biological treatment of highly saline wastewaters containing aromatic hydrocarbons. Acikgoz and Ozcan (2016) isolated a total of 103 halophilic *Archaea* from different parts of Turkey to study phenol biodegradation. The aromatic compound

phenol is known to be toxic produced after various industrial activities. The maximum phenol degradation capacity was obtained with the strain A235, among all strains studied on liquid and solid media with 20% (w/v) NaCl and phenol as the only carbon source.

The novel industrially important enzymes isolated and characterised from halophiles, which are stable in harsh conditions such as thermal, salt, alkaline and organic solvent stability, may well present advantages in different industrial processes (Souza 2010; Kumar et al. 2012). Although a review on halophilic proteins and their applications have been already reviewed by Calimlioglu and Arga (2016) in general, there are not enough studies on halophilic enzymes from microorganisms isolated from saltern areas in Turkey. As an example, extremely halophilic microorganism communities comprising of *Archaebacteria* and *Eubacteria* isolated in Sereflikochisar Salt Lake located in central Turkey were studied by Birbir and Sesal (2003). It was also demonstrated that a fairly wide diversity of halophilic species were found to produce industrial enzymes such as lipases, gelatinases, cellulases and β-galactosidases.

Another study was carried out on a thermostable amylase produced by moderately halophilic microorganism, namely, *Halomonas* sp. strain AAD21, isolated from the Camalti Saltern located in Izmir Province. On the basis of morphological and biochemical characteristics and 16S rRNA gene sequence analysis, the strain was assigned to the genus *Halomonas*. The strain was found to grow at wide salt concentration range of 3–20% (w/v) NaCl, with optimum of 10%. The optimum temperature and pH of the α-amylase were determined as 50 °C and 7.0, respectively. The α-amylase from *Halomonas* sp. AAD21 was found to be thermostable, as 70% of original enzyme activity was retained during 120 min of incubation at 90 °C, which claimed to be a good candidate for use in severe process conditions of starch hydrolysis or detergent industry (Uzyol et al. 2012).

Ozcan et al. (2009) screened as many as 118 halophilic archaeal strains in search of lipolytic activity, five of which were selected and further characterised to determine the effects of salt, temperature and pH at various ranges on the optimum esterase and lipase activities. The highest hydrolytic production was determined for the strains grown at a special medium containing 25% NaCl and 1% arabic gum. The maximum activity of esterase was observed at temperature 60–65 °C, pH 8–8.5 and at 3–4.5 M NaCl, while the highest activity of lipase was determined at temperatures between 45 and 65 °C, pH of 8.0, and NaCl range of 3.5–4 M, indicating the presence of the temperature-tolerant and salt-dependent archaeal lipolytic enzymes. The results also showed that the strains had a higher esterase activity compared to lipase activity.

A most recent study has been published on new bacterial sources of halophilic lipases. It has been highlighted on the Turkish and Spanish hypersaline biotopes to be a suitable source of halophilic microorganisms producing lipolytic enzymes, which are from two different points in salterns of Parque Natural de las Lagunas de La Mata y Torrevieja (Spain) and from Pamukkale (Turkey). Three strains growing at NaCl concentration greater than 15% were capable to synthesise lipolytic enzymes, though one of them identified as *Halomonas* sp. LM1C was demonstrated

to have high enzyme production levels. Subsequently this strain was used, and the highest lipase production was obtained at pH 6.9 and 21.6 °C. The optimum values for the enzyme-biocatalysed hydrolysis were determined as neutral pH and 29 °C. The extracellular lipase displayed a high salt tolerance, which claimed to pose the economic advantages in industrial applications (Gutiérrez-Arnillas et al. 2016).

8.7 Future Perspective

Industrial biotechnology is a key technology for future economic development. Thus, for developing countries such as Turkey, there is a need to expand the research in biotechnology field. Since Turkey owns different ecological areas, i.e. surrounded by seas, salt lakes and many hot springs with a broad microbial diversity including extremophiles, there are a great deal of opportunities for newly isolated microorganisms from extreme environments for their use in biotechnological applications. From this point of view, it seems that the search of extremophiles in the country is very recent, and this potential needs to be fully exploited.

There is a limited data on archaea isolated from extreme environments in Turkey, which need to be considered in many investigations. Particularly, their roles as source of enzymes from extremophile archaea (thermostable DNA polymerases, amylase, galactosidases and pullulanases) have a wide range of potential uses and also known to be very stable in organic solvents, providing an advantage in use for environmentally friendly processes. For instance, acidophilic archaea give a promise in mineral processing for the extraction of several metals such as gold and copper, as Turkey is known to possess gold and copper mines.

Recent advances in molecular genetic tools for extreme microorganisms lead to their use for metabolic engineering for the production of chemicals and fuels. The advantages and drawbacks of using extremophiles as industrial hosts need to be discussed further with perspectives on future developments in this emerging technology (Loder et al. 2017). Today, in most countries, there is a trend towards cheap, renewable and readily available biomass in the production of various chemicals utilising extremophiles and their enzymes. It is a fact that Turkey is one of the top agriculture-producing countries in the world. As a result, there are potential and existing applications of both thermophiles and thermostable enzymes on conversion of raw materials containing carbohydrate into the desired products in industrial biotechnology. Moreover, the studies on thermophiles have extended to energy biotechnology such as biofuel, biohydrogen and ethanol production. Microorganisms isolated can be explored for the production of next-generation biofuels by the use of the carbohydrate fraction in lignocellulosic material (Turner et al. 2007).

It has been already suggested that at higher temperatures the ethanol production would make the process design easy, and the engineered progeny of *Thermoanaerobacter mathranii*, *Thermoanaerobacterium saccharolyticum* and *Geobacillus thermoglucosidasius* now forms the platform for new biotechnology companies (Taylor et al. 2009; Olson et al. 2015).

On the other hand, the potentials of halophiles and their bioproducts have been extensively reviewed (Ventosa et al. 1998; Madern et al. 2000; Oren 2010; DasSarma and DasSarma 2017), as well as genetic tools for manipulation of moderately halophilic bacteria with promising applications in biotechnology (Vargas and Nieto 2004). Halophilic enzymes are important candidates for use in biotransformation reactions in harsh industrial environments such as cosmetic, agrochemical, textile, detergent, paper, fuel, energy and pharmaceutical industries, which all require the organic solvent addition, very high temperatures, high salt concentration, low water level, high pH levels, etc. Despite some studies which have been already performed on halophilic microorganisms in different regions of Turkey, halophiles still need a special interest in terms of isolation and characterisation of new species producing desirable biocatalysts and biomolecules to fulfill future biotechnological and industrial demands. Most recently, biosynthesis of nanoparticles using extreme microorganisms has emerged as rapidly developing research area in green nanotechnology in the world as forming an alternative for conventional chemical and physical methods, which should also be taken into consideration.

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