5

Geobacillus and *Anoxybacillus* spp. from Terrestrial Geothermal Springs Worldwide: Diversity and Biotechnological Applications

Armine Margaryan, Grigor Shahinyan, Pargev Hovhannisyan, Hovik Panosyan, Nils-Kåre Birkeland, and Armen Trchounian

Abstract

A large number of thermophilic representatives of the *Geobacillus* and *Anoxybacillus* genera have been isolated from geographically distant and physicochemically different environments, including high-, moderate-, and low-temperature habitats. However, terrestrial hot springs are the main habitats for *Geobacillus* and *Anoxybacillus* species. The members of these genera possess a variety of thermo-adaptive features that enable them to thrive at elevated temperatures. Due to their ability to withstand harsh environmental conditions, geobacilli and anoxybacilli are a valuable source for provision of thermostable enzymes, such as amylases, lipases, proteases, etc., and other components. Thermostable enzymes obtained from thermophilic bacilli have found a plethora of commercial applications due to their sturdiness and toughness in withstanding the heat generated in various biotechnological and industrial processes. This

A. Margaryan · A. Trchounian
Department of Biochemistry, Microbiology and Biotechnology, Yerevan State University, Yerevan, Armenia

Research Institute of Biology, Yerevan State University, Yerevan, Armenia

G. Shahinyan
Research Institute of Biology, Yerevan State University, Yerevan, Armenia

P. Hovhannisyan · H. Panosyan (✉)
Department of Biochemistry, Microbiology and Biotechnology, Yerevan State University, Yerevan, Armenia
e-mail: hpanosyan@ysu.am

N.-K. Birkeland
Department of Biological Sciences, University of Bergen, Bergen, Norway

D. Egamberdieva et al. (eds.), *Extremophiles in Eurasian Ecosystems: Ecology, Diversity, and Applications*, Microorganisms for Sustainability 8,
https://doi.org/10.1007/978-981-13-0329-6_5

chapter contains a review of studies of geobacilli and anoxybacilli from terrestrial geothermal springs worldwide with special emphasis on their distribution and diversity, ecological significance, adaptive mechanisms, enzymes, and biotechnological potential.

Keywords
Terrestrial geothermal springs · *Geobacillus* · *Anoxybacillus* · Thermostability · Thermostable enzymes · Extremophiles

5.1 Introduction

Terrestrial hot springs are manifestations of geological activity and represent aquatic microcosms that are formed by the emergence of geothermally heated groundwater from the Earth's crust (Mehta and Satyanarayana 2013b). Terrestrial hydrothermal springs represent extreme environments and have been found worldwide, like those in Yellowstone National Park, which harbor the closest relatives to the original organisms that lived on our planet. Finding these features on Mars (or any other planet) could have big implications for the question of extraterrestrial life (Van Kranendonk et al. 2017). Hence, the microbiota thriving in geothermal hot springs have been the subject of extensive research. Among the diversity of microbes harboring the hot springs in different parts of the world, members of the *Geobacillus* and *Anoxybacillus* genera were frequently isolated and extensively studied during the last decades. The members of *Geobacillus* and *Anoxybacillus* genera are thermophilic bacilli, which have adapted to grow optimally at temperatures ranging from 35 to 75 °C (Bergey et al. 2009). The ability of these microorganisms to grow at high temperatures has made them suitable objects for studying and understanding the thermostability mechanisms for the microbial adaptations to harsh conditions.

Thermophilic bacilli constitute valuable sources for various biotechnological products (Antranikian 2007; Satyanarayana et al. 2012). The members of the *Geobacillus* and *Anoxybacillus* genera have shown tremendous potential in biotechnology because of their ability to produce unique thermostable enzymes and proteins with high industrial and economical values (Antranikian 2007; Gurumurthy and Neelagund 2012). The recent interest in biotechnology, coupled with the discovery of novel thermophilic bacilli, has prompted studies on the utilization of thermophiles and their enzymes, such as amylase (Gurumurthy and Neelagund 2010, 2012, Rekadwad 2015, Acer et al. 2016), lipase (Balan et al. 2012; Mahadevan and Neelagund 2014; Ozdemir et al. 2015; Ay et al. 2011), protease (Hawumba et al. 2002; Zhu et al. 2007; Nakamichi et al. 2010), xylanase (Sunna et al. 1997; Kacagan et al. 2008; Ellis and Magnuson 2012; Inan et al. 2013), and cellulase (Ibrahim and El-diwany 2007; Padilha et al. 2015).

Enzymes from these microorganisms are in great demand as they are not usually denatured at high temperatures but are rather more active. These enzymes are also more resistant to chemical reagents and extreme pH values in comparison with their

mesophilic homologues (Synowiecki 2010; Pinzon-Martinez et al. 2010). Moreover, their thermostability is associated with higher biochemical reaction rates, lower viscosity, and less risk of contamination (Turner et al. 2007). All these factors have stimulated a renewed interest in the exploration of extracellular enzymatic activities of thermostable organisms.

The objective of this chapter is to review the findings of the diversity, thermostability mechanisms, and biotechnological applications of microbes belonging to genera *Geobacillus* and *Anoxybacillus* from different terrestrial geothermal springs worldwide.

5.2 Taxonomy and Species Diversity

The genera *Geobacillus* and *Anoxybacillus* of the phylum Firmicutes comprise a group of Gram-positive, endospore-forming, rod-shaped, chemoorganotrophic thermophilic bacteria, including obligate aerobes, denitrifiers, and facultative anaerobes that can grow over a temperature range of 35–75 °C. Their catabolic versatility, particularly in the degradation of starch, xylene, cellulose, and lipids, and rapid growth rates have raised their profile as organisms with high potential for industrial and biotechnological applications.

5.2.1 The Genus *Geobacillus*

The members of the genus *Geobacillus* were originally classified in the genus *Bacillus*, as thermophilic variants of *Bacillus* spp. For many years *Bacillus stearothermophilus* (Donk 1920) was the only obligate thermophilic species of the genus *Bacillus* with a validly published name. After 1980, additional thermophilic species were proposed based on phenotypic analyses of novel isolates. Subsequent 16S rRNA gene sequencing indicated that *B. stearothermophilus*, *Bacillus kaustophilus*, and *Bacillus thermoglucosidasius* formed a phylogenetic lineage that was distinct from other *Bacillus* spp. (Ash et al. 1991). The continued development of genetic tools to facilitate both fundamental investigations and metabolic engineering and accumulating evidence for clustering of many of the thermophiles in a separate subgroup (group 5) supported by 16S rRNA analysis led to their reclassification as a separate genus (Nazina et al. 2001). Nazina et al. (2001) proposed that the six species of that lineage, namely, *Bacillus stearothermophilus*, *B. kaustophilus*, *B. thermoglucosidasius*, *B. thermocatenulatus*, *B. thermoleovorans*, and *B. thermodenitrificans*, should be placed in a new genus, *Geobacillus*, with *G. stearothermophilus* as the type species and along with two novel species, *G. subterraneus* and *G. uzenensis*. *B. pallidus* (Scholz et al. 1987), *Saccharococcus caldoxylosilyticus* (Ahmad et al. 2000), and *B. vulcani* (Caccamo et al. 2000) were also transferred to the genus *Geobacillus* (Fortina et al. 2001; Banat et al. 2004; Nazina et al. 2004). Subsequently, six additional species, *G. toebii* (Sung et al. 2002), *G. gargensis* (Nazina et al. 2004), *G. debilis* (Banat et al. 2004), *G. lituanicus* (Kuisiene et al.

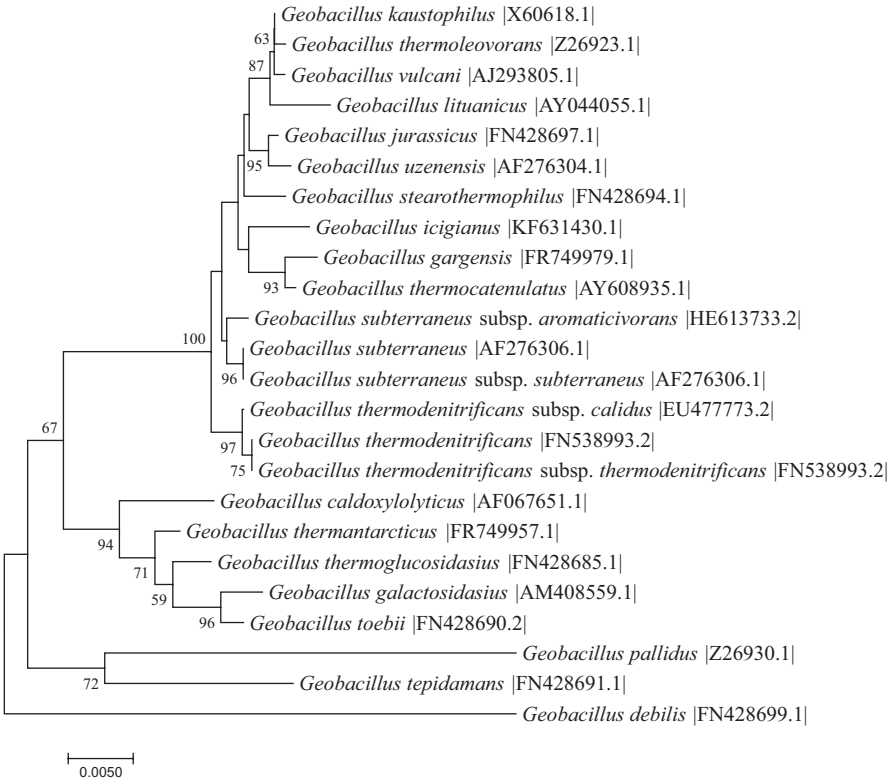

Fig. 5.1 Evolutionary relationships of species of the genus *Geobacillus*. The evolutionary history was inferred using the neighbor-joining method. The optimal tree with the sum of branch length = 0.17390653 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. Significant bootstrapping values (>59%) are shown on the nodes. The analysis involved 24 16S rRNA nucleotide sequences of the type strains of *Geobacillus* species, obtained from NCBI GenBank (accession numbers shown between bars). All positions containing gaps and missing data were eliminated. There were a total of 1218 positions in the final dataset. Evolutionary analyses were conducted in MEGA7

2004), *G. tepidamans* (Coorevits et al. 2012), and *G. jurassicus* (Nazina et al. 2005), have been described. Currently the genus *Geobacillus* includes 20 species and 4 subspecies (Bergey et al. 2009). Miñana-Galbis et al. (2010) proposed the further transfer of *Geobacillus pallidus* to the new genus *Aeribacillus*.

The evolutionary tree showing the phylogenetic relationships of *Geobacillus* species is presented in Fig. 5.1.

The majority of geobacilli strains grow in the temperature range 35–75 °C, with the optimum at 55–65 °C. Vegetative cells are rod-shaped and occur either singly or in short chains and are motile by means of peritrichous flagella, or they are nonmotile. The cell-wall structure is Gram-positive, but the Gram-stain reaction may vary. Endospores are ellipsoidal or cylindrical and located terminally or subterminally in

slightly swollen or non-swollen sporangia. Colony morphology and size are variable; pigments may be produced on certain media. They are aerobic or facultatively anaerobic anaerobic. Oxygen is the terminal electron acceptor, replaceable in some species by nitrate. They are neutrophilic. Growth occurs at pH 6.0–8.5, with optimal growth at pH 6.2–7.5. Growth factors, vitamins, NaCl, and KCl are not required by most species. Most species can utilize n-alkanes as carbon and energy sources. Most species produce acid but not gas from fructose, glucose, maltose, mannose, and sucrose. Catalase and oxidase reaction varies. Most species produce extracellular thermostable hydrolytic enzymes that have high potential of use in industry. The major cellular fatty acids are C15:0 iso, C16:0 iso, and C17:0 iso, which make up more than 60% of the total. The main menaquinone type is MK-7. The lowest level of 16S rRNA gene sequence similarity between all *Geobacillus* species is around 93%, which indicates that at least some species need to be reclassified at the genus level (Bergey et al. 2009). The average genome size for *Geobacillus* spp. ranges from 3.5 to 3.9 Mbp. The smallest genome was found in *G. kaustophilus* and the largest in *G. thermoglucosidasius*. This might reflect the additional coding requirements associated with anaerobic growth, additional CRISPR regions, as well as genes of unassigned function found between transposable elements in the genome of *G. thermoglucosidasius*. Despite the small genome, it was shown that the highest number of IS/transposable elements was present in the *G. kaustophilus* (Hussein et al. 2015).

Geobacillus species are widely distributed in nature, and being catabolically diverse, they are readily isolated from active communities growing in compost, hot springs, and deep geothermal sites, including oil wells and deep sediments. However, it has long been known that *Geobacillus* spp. can be isolated from a wide range of moderate- and low-temperature environments including temperate soils and have also been isolated from low-temperature environments such as the Bolivian Andes, deep seawater, and even the Mariana Trench (Hussein et al. 2015).

5.2.2 The Genus *Anoxybacillus*

Genus *Anoxybacillus* has only been described recently by Pikuta et al. (2000, 2003). Since then, the number of *Anoxybacillus* species has rapidly increased and now contains 22 validly described species and 2 subspecies. The following species of the genus have been reported up to date: *Anoxybacillus pushchinoensis*, *A. flavithermus* (Pikuta et al. 2000), *A. gonensis* (Belduz et al. 2003), *A. contaminans* (De Clerck et al. 2004), *A. voinovskiensis* (Yumoto et al. 2004), *A. kestanbolensis*, *A. ayderensis* (Dulger et al. 2004), *A. kamchatkensis* (Kevbrin et al. 2005), *A. amylolyticus* (Poli et al. 2006), *A. rupiensis* (Derekova et al. 2007), *A. bogrovensis* (Atanassova et al. 2008), *A. kamchatkensis* subsp. *asaccharedens* (Gul-Guven et al. 2008), *A. thermarum* (Poli et al. 2009), *A. eryuanensis*, *A. tengchongensis* (Zhang et al. 2011), *A. salavatliensis* (Cihan et al. 2011), *A. mongoliensis* (Namsaraev et al. 2010), *A. flavithermus* subsp. *flavithermus*, *A. flavithermus* subsp. *yunnanensis* (Dai et al. 2011), *A. caldiproteolyticus* (Coorevits et al. 2012), *A. tepidamans* (Coorevits et al. 2012),

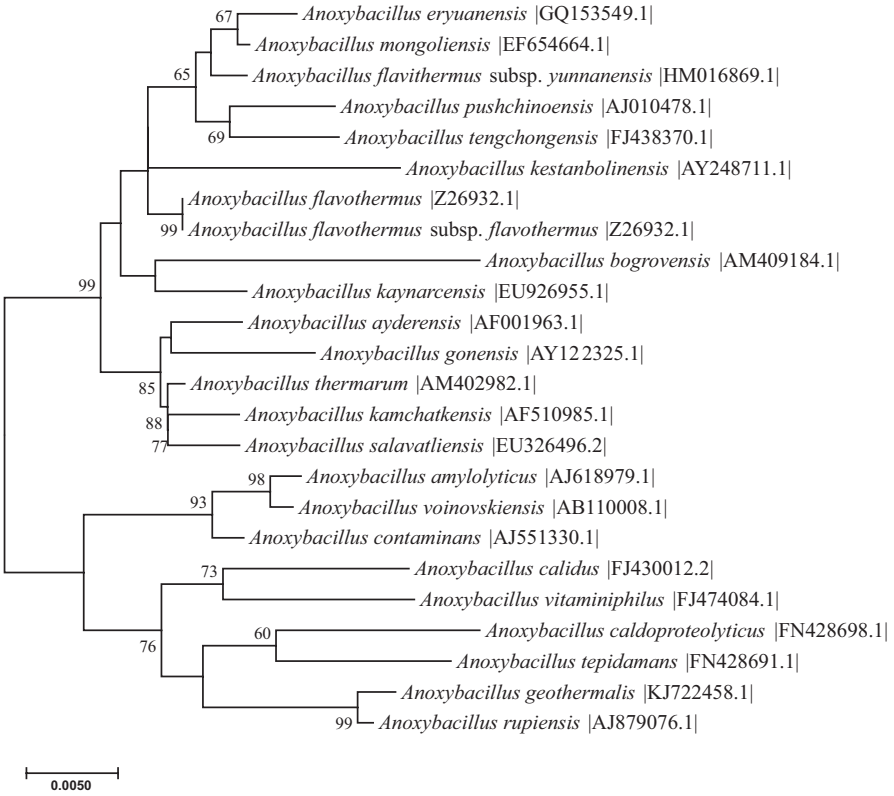

Fig. 5.2 Evolutionary relationships of species of the genus *Anoxybacillus*. The evolutionary history was inferred using the neighbor-joining method. The optimal tree with the sum of branch length = 0.18764567 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. Significant bootstrapping values (>60%) are shown on the nodes. The analysis involved 24 16S rDNA nucleotide sequences of the type strains of *Anoxybacillus* species, obtained from NCBI GenBank. All positions containing gaps and missing data were eliminated. There were a total of 1031 positions in the final dataset. Evolutionary analyses were conducted in MEGA7

A. kaynarcensis (Inan et al. 2013), *A. vitaminiphilus* (Zhang et al. 2013), *A. calidus* (Cihan et al. 2014), and *A. geothermalis* (Filippidou et al. 2016). *A. kaynarcensis* and *A. kamchatkensis* subsp. *asaccharedens* are still not included in the validation list.

Most of the species belonging to genus *Anoxybacillus* were found to be a homogeneous phylogenetic group of thermophilic bacilli with high 16S rRNA gene sequence similarity values. A tree showing the phylogenetic relationships of species of the genus *Anoxybacillus* is shown in Fig. 5.2.

As it can be deduced from the genus name (“anoxybacillus” means small rod living without oxygen), the members of the genus *Anoxybacillus* are aerotolerant

anaerobes or facultative anaerobes (Pikuta et al. 2000). *A. pushchinoensis*, the type strain of this genus, was first described as obligate anaerobe (Pikuta et al. 2000) but was later described as an aerotolerant anaerobe (Pikuta et al. 2003).

The majority of *Anoxybacillus* species are moderate thermophiles (grow in the temperature range 30–75 °C, with the optimum at 50–62 °C). Vegetative cells are rod-shaped or straight or slightly curved, sometimes with angular division and Y-shaped cells, often in pairs or short chains, with rounded ends. The cells are motile or nonmotile. Endospores are round, oval, or cylindrical and have a terminal location. Colony morphology and size are variable. Most of the species produce cellular carotenoid like pigments, which yields yellow colonies. They are catalase-variable. Many members of the genus are alkaliphilic, but most of the species can grow at neutral pH. Only *A. amylolyticus* grows optimally at slightly acidic conditions (pH 5.6). *Anoxybacillus* species are chemoorganotrophic, with a fermentative or aerobic respiration metabolism. They can use oxygen or nitrate as electron acceptors, and in the absence of electron acceptors, they perform fermentation by the Embden-Meyerhof-Parnas pathway.

Many species produce a variety of thermostable enzymes, such as amylase (Poli et al. 2006; Baltas et al. 2016), glucosidase (Cihan et al. 2011), esterase (Shahinyan et al. 2017; Chis et al. 2013), proteinase (Matpan Bekler et al. 2015; Nakamichi et al. 2010), and xylanase (Inan et al. 2013; Ellis and Magnuso 2012; Kacagan et al. 2008).

Most species of the genus have been isolated from hot springs. They have been found also in geothermal soils, manure, hydrothermal vents, etc. (Bergey et al. 2009).

5.3 Distribution of *Geobacillus* and *Anoxybacillus* in Terrestrial Hot Springs

Since Thomas Brock made the remarkable discovery in 1966 that microorganisms were growing in the boiling hot springs of Yellowstone National Park, the search for thermophiles in terrestrial hot springs has increased. Terrestrial hot springs are created by the emergence of geothermally heated groundwater from the Earth's crust (Mehta and Satyanarayana 2013a, b). Thermophilic microbes have been discovered in geothermal springs all over the world, including areas in Asia, America, Kamchatka, Iceland, New Zealand, Italy, China, etc.

Thermophilic representatives of the *Geobacillus* and *Anoxybacillus* genera have been recovered from a variety of environments, including high-, moderate-, and low-temperature environments. However, terrestrial hot springs are the main habitats for *Geobacillus* and *Anoxybacillus* species (Fig. 5.3). An overview of the various *Geobacillus* and *Anoxybacillus* species isolated from terrestrial hot springs is given in Tables 5.1 and 5.2.

Fig. 5.3 Geographical distribution of sites from where *Geobacillus* and *Anoxybacillus* species have been isolated. Green and purple circles represent *Geobacillus* and *Anoxybacillus* isolates, respectively. Each circle denotes a published report that describes one or several strains

5.4 Adaptations of Growth at High Temperatures

Thermophilic bacilli, growing at high temperatures, have developed different mechanisms to survive in these extreme environments. Understanding the adaptations that enable thermophilic organisms to survive at extreme temperatures is a challenge that has interested researchers since 1897, and a vast amount of literature exists regarding this issue (England et al. 2003). The main mechanistic determinants of thermoadaptation in bacilli are adaptation of membrane phospholipid composition, synthesis of heat shock proteins (HSPs), and enzyme adaptation to give molecular stability as well as structural flexibility. The high GC content in the genome of the thermophiles also contributes to their thermoadaptation (Chakravorty and Petra 2013).

5.4.1 Adaptation of Membrane Phospholipid Composition at High Temperatures

It has been shown that the lipids isolated from a psychrophilic (*Psychrobacter* sp.), mesophilic (*Escherichia coli*), and thermophilic (*G. stearothermophilus*) bacteria are different depending on the bacterial growth temperature. With increasing growth temperature, bacteria reduce the number of unsaturated bonds or increase the degree of branching in their lipid acyl chains (van de Vossenberg et al. 1995). Thus, the lipid of the cytoplasmic membrane of *Psychrobacter* sp. (optimal growth temperature 21–29 °C) is mainly represented by monounsaturated (93%) and short-chain lipids. The monounsaturated and short-chain lipids compose 32% of the cytoplasmic membrane lipids in *E. coli* (optimal growth temperature 37–42 °C), whereas the

Table 5.1 *Geobacillus* species recovered from hot springs worldwide

Species	Source	Place	Temp. range (°C)	Opt. temp. (°C)	pH range	Opt. pH	Comments	References
G. gargensis	Garga hot spring, in the valley of the river Barguzin	Baikal region, Russia	45–70	60–65	5.5–8.5	6.5–7.0	–	Nazina et al. (2004)
G. stearothermophilus			ND	ND	ND	ND	The draft genome sequence is available (GenBank AN:JQCS00000000 and JPYV00000000)	Rozanov et al. (2014)
	Hot springs in Yellowstone National Park	USA	ND	55–65	ND	ND	DNA polymerase has been characterized	Stenesh and McGowan (1977) and Zeigler (2001)
	Hot spring in Chiang Mai	Thailand	ND	65	ND	ND	Produces an extracellular superoxide dismutase (SOD)	Sookkheo et al. (2002)
	Hammam Pharaon hot spring	Egypt	ND	70	ND	ND	Producing thermostable cellulases	Ibrahim and El-diwany (2007)
	Hot spring areas in Bulgaria	Bulgaria	50–82	55	5.5–8.5	7.0	Producing thermostable gellan lyase	Derekova et al. (2006)
	Dikili-Bergama Kaynarca hot spring in Izmir	Turkey	40–70	55	5.5–8.5	7.0	High xylanase and arabinofuranosidase activities	Canakci et al. (2007)
	Savusavu hot spring	Fiji	ND	ND	5–8	ND	High cadmium ion adsorption potential	Narayan et al. (2008)
	Dalupirip hot spring	Benguet, Philippines	ND	60	ND	7.0	Xylan-degrading ability	Daupan and Rivera (2015)

(continued)

Table 5.1 (continued)

Species	Source	Place	Temp. range (°C)	Opt. temp. (°C)	pH range	Opt. pH	Comments	References
	Soldhar hot spring	Garhwal Himalaya, India	40–90	65–70	4–11	6–8	–	Sharma et al. (2009)
	Arzakan hot geothermal mineral spring	Kotayk province, Armenia	37–70	55	6.5–8.5	ND	–	Panosyan (2010), Panosyan and Birkeland (2014), Hovhannisyan et al. (2016), and Panosyan (2017)
	Jermuk hot spring	Vayots Dzor province, Armenia	ND	ND	ND	ND	Active amylase producer	Vardanyan et al. (2015) and Hovhannisyan et al. (2016)
G. thermodenitrificans	Hammam Pharaon hot spring	Egypt	ND	70	ND	ND	Producing thermostable cellulases	Ibrahim and El-diwany (2007)
	Alangullu, Omerbeyli, and Camkoy Camur hot springs	Aydin, Turkey	40–75	55	5.5–8.5	7.0	Showed high xylanase and arabinofuranosidase activities	Canakci et al. (2007)
	Tattapani hot spring	Northwest Himalayas, India	60–80	60	ND	7.0	Produced a thermotolerant cellulose GenBank AN:KP842609	Priya et al. (2016)

	Arzakan hot geothermal mineral spring	Kotayk province, Armenia	37–70	55	6.5–8.5	ND	–	Panosyan (2010), Panosyan and Birkeland (2014), Hovhannisyan et al. (2016), and Panosyan (2017)
G. thermoparaffinivorans	Badekbek hot spring	Benguet, Philippines	ND	60	ND	7.0	Xylan-degrading ability	Daupan and Rivera (2015)
G. thermoglucosidasius	Obsidian hot spring, Yellowstone National Park	Montana, USA	55–75	65	5.8–8.0	7.5	Complete genome is reported (GenBank AN:CP002835)	Brumm et al. (2015)
	North Shuna hot spring	Jordan	40–80	60	6–9	6–8	Exhibited high hydrolytic activities	Obeidat et al. (2012)
G. thermoleovorans	Ulu Slim hot spring	Malaysia	ND	ND	ND	ND	Complete genome is reported	Muhd Sakaff et al. (2012)
	Hot spring of the Waimangu Volcanic Valley	New Zealand	ND	70	ND	7.0	Producing thermostable alpha amylase	Uma Maheswar Rao and Satyanarayana (2007)
	Aguas Calientes geothermal spring	Amazon rainforest, Peru	50–70	ND	ND	7.4	Characterized with high cellulase activity	Cortez et al. (2016)
	Hot spring in Kobe	Japan	50–75	60	3.0–11	7.0	Produce thermoactive xylanases	Sunna et al. (1997)
	Badekbek hot spring	Benguet, Philippines	ND	60	ND	7.0	Showed xylan-degrading ability	Daupan and Rivera (2015)

(continued)

Table 5.1 (continued)

Species	Source	Place	Temp. range (°C)	Opt. temp. (°C)	pH range	Opt. pH	Comments	References
G. thermocatenulatus	El Tatio geyser field and Liquiñe hot springs	Chile	ND	60	ND	ND	Phytase activity in crude protein extracts	Jorquera et al. (2018)
G. icigianus	Hot spring of the Valley of Geysers	Kamchatka, Russia	50–75	60–65	5–9	6.5–7.0	The draft genome sequence is available (GenBank AN: JPYA00000000)	Bryanskaya et al. (2015)
G. subterraneus subsp. *aromaticivorans*	Guclukonak hot spring	Sirnak, Turkey	30–65	60	5.5–10	9.0	The isolate hydrolyses lipase, ONPG, phosphatase, urease, oxidase, gelatin, and starch	Poli et al. (2012)
G. caldoxylosilyticus	Fosso Bianco hot springs	Bagni di Filippo, Mount Amiata, Tuscany, Italy	45–70	55–60	ND	7.0	Able to grow in the presence of Hg(II) (>1 mM)	Chatziefthimiou et al. (2007)
	Selayang hot spring	Malaysia	45–85	55	5.0–9.0	6.5	Able to reduce toxic chromium (VI) to non-harmful of chromium (III)	Che Ibrahim and Wan Ahmad (2017)
	Jermuk hot spring	Vayots Dzor province, Armenia	ND	ND	ND	ND	Active amylase producer	Vardanyan et al. (2015) and Hovhannisyan et al. (2016)

G. kaustophilus	Aguas Calientes geothermal spring	Amazon rainforest, Peru	50–70	ND	ND	7.4	High cellulase activity	Cortez et al. (2016)
	Dalupirip hot spring	Benguet, Philippines	ND	60	ND	7.0	Xylan-degrading ability	Daupan and Rivera (2015)
	Soldhar hot spring	Garhwal Himalaya, India	40–90	65–70	4–11	6–8	–	Sharma et al. (2009)
G. pallidus	Tanjung Sakti hot spring	South Sumatera, Indonesia	ND	ND	ND	ND	–	Yohandini et al. (2015)
G. pallidus	Hammamt Al-Burbita, Afra hot springs, Ma'in hot springs	Jordan	ND	ND	ND	ND	–	Al-Batayneh et al. (2011)
G. toebii	Arzakan hot geothermal mineral spring	Kotayk Province, Armenia	37–70	55	6.5–8.5	ND	–	Panosyan (2010), Panosyan and Birkeland (2014), Hovhannisyan et al. (2016), and Panosyan (2017)
Geobacillus spp.	Hammamat Afra, Jordan Himma, Zara-Bani Hamida, Ma'in-Roman hot spring	Jordan	40–85	65	4–11	6–8	High hydrolytic activities	Obeidat et al. (2012)
	Soldhar hot spring	Garhwal Himalaya, India	40–90	65–70	4–11	6–8	–	Sharma et al. (2009)

(continued)

Table 5.1 (continued)

Species	Source	Place	Temp. range (°C)	Opt. temp. (°C)	pH range	Opt. pH	Comments	References
	Irde geothermal spring	Konkan region, Southern India	40–95	60	ND	8.0	Active thermostable lipase and amylase producer	Mahadevan and Neelagund (2010, 2014)
	Tattapani hot spring	Azad Kashmir, Pakistan	45–75	65	5.5–8.5	7.0	Produced significant amount of industrially important enzymes, i.e., extracellular α-amylase, CMCase, FPase, xylanase, protease and lipase, and intracellular CMCase and FPase	Zahoor et al. (2016)
	Tapovan hot spring	Chamoli, Uttarakhand, India	40–90	70	6–8.5	7–8	Active α-amylase producer. High amylase activity at 80 °C and pH 8.0	Jugran et al. (2015), Arya et al. (2015)
	Tengchong hot spring	China	ND	60–65	ND	ND	α-amylase producer	Wang et al. (2011b)
	Double Hot Springs, Nevada	Nevada, USA	ND	ND	ND	ND	Draft genome is reported (GenBank AN: SAMN0017395)	De Maayer et al. (2014)
	Larijan hot spring	Iran	40–80	65	ND	6.8	Producing alpha amylase with 52 kDa molecular mass	Mollania et al. (2010)
	Hot spring in Rosario de la Frontera	Salta, Argentina	ND	ND	ND	ND	Whole-genome shotgun project is reported (GenBank AN: LDNZ00000000)	Ortiz et al. (2015)

	Alangullu, Omerbeyli, and Camkoy Camur hot springs	Aydin, Turkey	35–65	55	5.5–9	7.0	Showed high xylanase and arabinofuranosidase activities	Canakci et al. (2007)
	Buranga hot springs	Western Uganda, Africa	37–72	60–62	5–10	7.5–8.5	Producing thermostable protease	Hawumba et al. (2002)
	Arzakan hot geothermal mineral spring	Kotayk province, Armenia	37–70	55	6.5–8.5	ND	–	Panosyan (2010), Panosyan and Birkeland (2014), Hovhannisyan et al. (2016), and Panosyan (2017)
	Tatev geothermal spring	Syunik province, Armenia	45–70	65	6–9	7.0	Lipase encoding genes have been reported	Shahinyan et al. (2017)

ND not determined, – data not available

Table 5.2 *Anoxybacillus* species recovered from hot springs worldwide

Species	Source	Place	Temp. range (°C)	Opt. temp. (°C)	pH range	Opt. pH	Comments	References
A. rupiensis	Unnamed hot spring	Rupi basin, Bulgaria	35–67	55	5.5–8.5	6.0–6.5	Strictly aerobic, producing amylolytic enzymes	Derekova et al. (2007)
	Arzakan hot geothermal mineral spring	Kotayk province, Armenia	ND	ND	ND	ND	Active amylase producer	Hovhannisyan et al. (2016), Vardanyan et al. (2015), Panosyan (2017)
	Karvachar and Zuar geothermal springs	Nagorno-Karabakh						
	Tanjung Sakti hot spring	South Sumatera, Indonesia	ND	ND	ND	ND	–	Yohandini et al. (2015)
A. tepidamans	Geothermal heated soil	Yellowstone National Park, USA	39–67	55	6.0–9.0	7.0	Covered with an oblique S-layer lattice, composed of identical S-layer glycoprotein protomers	Schäffer et al. (2004)
A. voinovskiensis	Voinovskie hot spring	Kamchatka, Russia	30–64	54	7.0–8.0	7.0–8.0	–	Yumoto et al. (2004)
A. kaynarcensis	Kaynarca hot spring	Izmir, Turkey	35–70	60	6.0–10.0	7.0	Active alkaline xylanase producer	Inan et al. (2013)
A. flavithermus	Tanjung Sakti hot spring	South Sumatera, Indonesia	ND	ND	ND	ND	–	Yohandini et al. (2015)
	Unnamed hot spring	Northern Island of New Zealand	30–72	60–65	5.5–9.0	7.0	Produces a carotenoid	Heinen et al. (1982)
	Savusavu hot spring	Fiji	ND	ND	5–8	ND	Showed gelatinase activity	Narayan et al. (2008)
	Al-Ain Alhara thermal hot spring	Gazan, Saudi Arabia	ND	55	ND	7.0	The draft genome sequence is reported (GenBank AN: APCD00000000)	Khalil et al. (2015)

	Mickey Hot Springs area of the Alvord Basin hydrothermal system	Oregon, USA	ND	65	ND	9.0	Extracellular xylanase enzymes producer	Ellis and Magnuson (2012)
	Dalupirip hot spring	Benguet, Philippines	ND	60	ND	7.0	Degrading xylan	Daupan and Rivera (2015)
	Hot spring in Tăşnad	Satu Mare county, Romania	40–70	50–60	6.5–8.5	7.5	Thermostable esterase/lipase with molecular weight of 28.03 kDa was characterized	Chis et al. (2013)
	The New Lorne bore thermal basin	Great Artesian Basin, Australia	50–65	ND	ND	ND	Can use Fe(III) as electron acceptor and yeast extract as carbon source	Ogg et al. (2013)
	Karvachar geothermal spring	Nagorno-Karabakh	40–70	60	7.0–11.0	9.0–10.0	GDSL family lipase encoding genes have been reported. Active thermostable alpha amylase producer	Shahinyan et al. (2017), Panosyan et al. (2014), Panosyan (2017)
	Omer hot spring	Afyonkarahisar, Turkey	25–85	55	5.0–10.0	6.0	Thermostable α-amylase was characterized, and some of its industrial applications were examined	Agüloğlu Fincan et al. (2014), Ozdemir et al. (2015)
	Hammam Pharaon hot spring	Egypt	ND	70	ND	ND	Producing thermostable cellulases	Ibrahim and El-diwany (2007)
	Fosso Bianca hot springs	Bagni di Filippo, Mount Amiata, Tuscany, Italy	45–65	55–60	ND	7.0	Fosso Bianca hot springs are naturally enriched with mercury of geological origin. Isolates able to grow in the presence of Hg(II) (40 μM–1 mM)	Chatziefthimiou et al. (2007)
A. contaminans			45–65	55–60	ND	7.0		

(continued)

Table 5.2 (continued)

Species	Source	Place	Temp. range (°C)	Opt. temp. (°C)	pH range	Opt. pH	Comments	References
A. flavithermus subsp. *yunnanensis*	Unnamed hot springs	Yunnan, China	30–66	60	5.5–10.0	7.0–7.5	Produce thermostable β-glucosidase. Optimal enzyme activity at 60 °C and pH 7.0	Dai et al. (2011), Liu et al. (2017)
A. bogrovensis	Geothermal spring	Sofia, Bulgaria	40–69	65	6.0–10.0	8.0	Hydrolysis of starch and gelatin	Atanassova et al. (2008)
A. eryuanensis	Eryuan hot spring	Yunnan, China	35–75	55	7.0–11.0	8.0	–	Zhang et al. (2011)
A. mongoliensis	Tsenkher hot spring	Central Mongolia	35–75	60	5.0–10.8	8.0	Produced thermostable alkaline subtilisin like serine proteinase	Namsaraev et al. (2010)
A. tengchongensis	Tengchong hot spring	Yunnan, China	30–75	50	7.0–11.0	8.5	Hydrolysis of starch and gelatin	Zhang et al. (2011)
A. gonensis	Gonen hot springs	Balikesir, Turkey	40–70	55–60	6.0–10.0	7.5–8.0	Produces xylose isomerase, carboxylesterase, and fructose-1,6-bisphosphate aldolase	Belduz et al. (2013), Lim et al. (2015)
A. ayderensis	Ayder hot springs	Rize, Turkey	30–70	50	6.0–11.0	7.5–8.5	Genes encoding various glycoside hydrolases are reported in the genome of the strain	Dulger et al. (2004), Belduz et al. (2015)
A. kestanbolensis	Kestanbol hot spring	Canakkale, Turkey	40–70	50–55	6.0–10.5	7.5–8.5	–	

A. kamchatkensis	Hot spring in the Valley of Geysers	Kamchatka, Russia	38–67	60	5.7–9.9	6.8–8.5	–	Kevbrin et al. (2005)
	Hot spring	Indonesia	ND	ND	ND	ND	This whole-genome shotgun project has been deposited (DDBJ/EMBL/ GenBank AN: ALJT00000000)	Lee et al. (2012)
	Los Baños hot spring	Mexico	37–60	50–70	6.0–11.0	6.5–7.5	Produces thermostable lipases, proteases, and amylases	Pinzón-Martínez et al. (2010)
	Kuala Woh hot spring	Malaysia	25–60	50–60	5–10	ND	Thermostable lipase producer	Olusesan et al. (2009)
	Dongda hot spring	Xi'an, Shaanxi province, China	ND	ND	ND	ND	Producing Cyclo(Gly-l-Pro) 20, which are immunomodulators of carp	Wang et al. (2011a)
A. kamchatkensis subsp. *asaccharedens*	Talisdere hot spring	Batman, Turkey	35–65	55	5.5–9.5	7.5	Not consuming sugars and carbohydrates as carbon source	Gul-Guven et al. (2008)
A. thermarum	Euganean hot spring	Abano Terme, Padova, Italy	55–67	65	6.0–7.5	7.2	Draft genome sequence is reported	Poli et al. (2009)
A. vitaminiphilus	Puge hot spring	Puge county, Sichuan province, China	38–66	57–60	6.0–9.3	7.0–7.5	A distinctive characteristic of this isolate was its extreme reliance on vitamin mixture or yeast extract for growth	Zhang et al. (2013)
A. suryakundensis	Surya Kund hot spring	Jharkhand, India	40–60	55	5.5–11.5	7.5	Draft genome sequence is reported. Hydrolysis of gelatin, starch, esculin, and DNA	Deep et al. (2016)

(continued)

Table 5.2 (continued)

Species	Source	Place	Temp. range (°C)	Opt. temp. (°C)	pH range	Opt. pH	Comments	References
Anoxybacillus spp.	Dargeçit hot spring	Turkey	30–65	60	5.5–10.0	7.0–7.5	Exhibited high alpha amylase activity (2668.4–3627 U/mL)	Acer et al. (2015)
	Seferihisar Karakoc hot spring	Turkey	ND	55	ND	7.4	Producing thermostable carboxylesterase	Ay et al. (2011)
	North Shuna hot spring	Jordan	ND	ND	ND	ND	–	Al-Batayneh et al. (2011)
	Sungai Klah and Dusun Tua hot springs	Malaysia	30–65	55	6.0–10.0	7.0	Strains SK3-4 and DT3-1 were able to degrade pullulan and to produce maltotriose and glucose, respectively, as their main end products	Chai et al. (2012)
	Hot spring in Beppu city	Oita, Japan	40–70	60	5.5–10.0	7.5–8.0	Producing thermostable protease	Nakamichi et al. (2010)

ND not determined, *AN* accession number, – data not available

cytoplasmic membrane lipids of *G. stearothermophilus* (optimal growth temperature 60–70 °C) are composed of saturated and branched acyl chains (80% of total lipids) (van de Vossenberg et al. 1995).

The thermophilic bacilli differ from mesophilic ones also in the fatty acid and polar headgroup compositions of their phospholipids. Hence, the major cellular fatty acid components of *Geobacillus* species following incubation at 55 °C are iso-$C_{15:0}$ (20–40%, mean 29%), iso-$C_{16:0}$ (6–39%, mean 25%), and iso-$C_{17:0}$ (7–37%, mean 19.5%), which account for 60–80% of the total (Nazina et al. 2001). The high levels of is-$C_{15:0}$ and iso-$C_{17:0}$ are also found in *Anoxybacillus* species (Dulger et al. 2004). It has been shown that the major fatty acid patterns in *G. toebii*, *G. subterraneus* subsp. *aromaticivorans*, *G. icigianus*, *A. bogrovensis*, and *A. suryakundensis* are iso-$C_{15:0}$>iso-$C_{17:0}$>anteiso-$C_{17:0}$ (Atanassova et al. 2008; Poli et al. 2012; Deep et al. 2016; Cihan et al. 2014; Bryanskaya et al. 2015). The membrane fatty acid patterns in *Bacillus* species are mainly represented by iso-$C_{15:0}$ and anteiso-$C_{15:0}$ in contrast with *Geobacillus* and *Anoxybacillus* species growing at higher temperatures (>50 °C) (Table 5.3). The acyl chains such as iso-$C_{17:0}$ have higher melting point than other acyl chains, which explains its synthesis at maximum growth temperatures, whereas iso-$C_{15:0}$ is predominating at minimum growth temperature.

Llarch et al. (1997) showed that any potential distinctions between the rather variable fatty acid profiles of *Geobacillus* species and *Bacillus* species are largely lost when strains of each group are incubated at the same temperature, clearly underpinning their role in thermoadaptation.

More detailed studies of the effect of temperature on the membrane composition of *G. stearothermophilus* showed that ratio of phosphatidylglycerol (PG) and cardiolipin (CL), which comprise about 90% of the membrane phospholipids, is changed at different growth temperatures. The PG content increases at the expense of the CL content at the high temperatures. The acyl-chain composition of all the membrane lipids also changes; the longer, saturated-linear, and iso-fatty acids with relatively high melting points increase in abundance, and anteiso-fatty acids and unsaturated components with lower melting points decrease (Tolner et al. 1997). Nicolaus et al. (1995), reclassifying some of the *Bacillus* species, showed that the strains tentatively identified as *Bacillus* showed increased phosphoglycolipid contents with increased growth temperature, at the expense of phosphoaminolipid and phospholipids. As a result, the organism is able to maintain nearly constant membrane fluidity across its whole growth temperature range; this has been termed homeoviscous adaptation (a homeostatic process that regulates the viscosity of membrane lipids). An alternative theory, homeophasic adaptation (adaptation of the cell membrane lipid composition), considers that maintenance of the liquid-crystalline phase is more important than an absolute value of membrane fluidity in bacteria (Tolner et al. 1997).

Table 5.3 The major cellular fatty acid composition of *Geobacillus* and *Anoxybacillus* species

Species	Temperature growth range (optimum) in °C	Major cellular fatty acids (>10%)	References
G. gargensis	45–70 (60–65)	Iso-$C_{15:0}$>iso-$C_{16:0}$>iso-$C_{17:0}$	Nazina et al. (2004)
G. icigianus	50–75 (60–65)	Iso-$C_{15:0}$>iso-$C_{17:0}$>anteiso-$C_{17:0}$	Bryanskaya et al. (2015)
G. subterraneus subsp. *aromaticivorans*	30–65 (60)	Iso-$C_{15:0}$>iso-$C_{17:0}$>anteiso-$C_{17:0}$	Poli et al. (2012)
G. stearothermophilus	37–65 (ND)	Iso-$C_{15:0}$>iso-$C_{17:0}$>anteiso-$C_{17:0}$	Nazina et al. (2001)
G. thermoglucosidasius	37–68 (ND)	Iso-$C_{17:0}$>iso-$C_{15:0}$>anteiso-$C_{17:0}$>iso-$C_{16:0}$	
G. uzenensis	45–65 (55–60)	Iso-$C_{17:0}$>iso-$C_{15:0}$>anteiso-$C_{17:0}$>iso-$C_{16:0}$	
G. toebii	45–70 (60)	Iso-$C_{15:0}$>iso-$C_{17:0}$	Cihan et al. (2014)
A. kaynarcensis	35–70 (60)	Iso-$C_{15:0}$>iso-$C_{17:0}$	Inan et al. (2013)
A. bogrovensis	40–69 (65)	Iso-$C_{15:0}$>iso-$C_{17:0}$>anteiso-$C_{17:0}$	Atanassova et al. (2008)
A. suryakundensis	40–60 (55)	Iso-$C_{16:0}$>iso-$C_{15:0}$>anteiso-$C_{17:0}$>iso-$C_{17:0}$	Deep et al. (2016)
A. pushchinensis	37–66 (62)	Iso-$C_{15:0}$> $C_{16:0}$> $C_{18:0}$	Pikuta et al. (2000)
A. rupiensis	35–67 (55)	Iso-$C_{15:0}$>iso-$C_{17:0}$	Cihan et al. (2014)
A. flavithermus	30–72 (60)	Iso-$C_{15:0}$>iso-$C_{17:0}$>$C_{16:0}$	
A. kamchatkensis	38–67 (60)	$C_{16:}$0>iso-$C_{16:0}$>anteiso-$C_{17:0}$	
A. calidus	35–70 (55)	Iso-$C_{15:0}$>iso-$C_{17:0}$>iso-$C_{16:0}$	

5.4.2 Heat Shock Proteins

Although a wide variety of survival strategies are deployed when cells are exposed to environmental challenges such as heat stress, synthesis of the effector proteins generally referred to as heat shock proteins (HSPs) is increased. HSPs are diverse in structure and function and are usually classified based on their subunit molecular weights. Classes that occur in microorganisms and in the majority of thermophiles include Hsp100, Hsp90, Hsp70, Hsp60, and the small HspS (Trent 1996). Most of these proteins function as molecular chaperones, catalyzing the refolding of denatured proteins, assisting the folding of newly synthesized proteins, as well as assisting in protein translocation across membranes and assembly/disassembly of protein complexes (Chang et al. 2008).

The 70-kDa heat shock proteins (Hsp70s) are highly conserved and are ATP dependent. Together with J-domain ATPase-activating proteins or nucleotide exchange factors, Hsp70s bind and release their substrates in ATP-driven cycles (Chang et al. 2008; Goh et al. 2014). Hsp70s chaperone family proteins, DnaK (GkDnaK) from *G. kaustophilus* (Chang et al. 2008), *G. thermoleovorans* (Graham et al. 2005), *G. thermoglucosidasius* (Brumm et al. 2015), *Geobacillus* sp. (Shih and Pan 2011), and *Anoxybacillus* sp. (Goh et al. 2014), have been identified and characterized.

Besides Hsp70s, the low-molecular-weight Hsp20 and Hsp33 proteins from *G. thermoglucosidasius* strain C56-YS93 have been described (Brumm et al. 2015). Proteome analysis of *G. thermoleovorans* strain T80 revealed the presence of sigma factors, such as σ^A, which initiates transcription of the heat shock operons controlled by the HRCA-CIRCE complex. This operon encodes some of the proteins involved in heat shock response, such as GroEL (Hsp60), GroES (Hsp10), and peptidyl-prolyl cis-trans isomerase (Graham et al. 2005).

The heat shock protein Hsp70 (DnaK) in *Anoxybacillus* works not only in the presence of ATP but also in cooperation with Hsp40 (DnaJ, J-protein). The genes encoding Hsp70 and Hsp40 proteins are located near each other. Other proteins related to temperature adaptations such as GroEL (Hsp60) and its co-chaperonin GroES (Hsp10), a few small Hsp20 molecular chaperones, Hsp33, and ClpC (Hsp100) and its related Clp-protease were identified in the genomes of many species of *Anoxybacillus* (Goh et al. 2014).

5.4.3 Protein and Enzyme Adaptation

Thermophilic bacilli, under constant threat of temperature-induced damage, maintain the stability and functionality of their proteins and enzymes by changing the ratio of charged to uncharged amino acids, increased ionic interactions and hydrogen bonding, metal coordination and the compactness of their proteins, and the preference of certain amino acids (Scandurra et al. 1998).

Lobry and Chessel (2003) reported that larger amounts of Ala, Gly, Ser, Asp, and Glu and smaller amounts of Cys in the transmembrane proteins of thermophiles have significant roles for their protein thermostability. Change in amino acids from Lys to Arg, Ser to Ala, Gly to Ala, Ser to Thr, and Val to Ile has been observed in comparison with mesophilic versus thermophilic organisms (Scandurra et al. 1998; Wang et al. 2015). For example, in *G. stearothermophilus*, it was reported that Gly is preferred over Ile and Ala over Tyr (Trivedi et al. 2006). Schneider et al. (2002) studied sequence differences between predicted transmembrane helices in the genomes of thermophilic and mesophilic membrane proteins. They observed a striking depletion of Cys residues in thermophiles and an increase in Gly, Ser, and Ala pair motifs, suggesting a preference for the packing of small residues. The integral membrane proteins of thermophiles have lower amounts of Glu, Lys, and Asp residues, as a mode of adaptation to increased temperature (Lobry and Chessel 2003).

Pertaining to secondary and three-dimensional structure, thermostable proteins have high levels of α-helical and β-sheet content (Chakravorty and Patra 2013). The thermostable lipases from *Geobacillus* and *Anoxybacillus* contain terminal α-helices and a central β-sheet (Arpigny and Jaeger 1999; Shahinyan et al. 2017) possibly contributing to its thermostability.

Sawle and Ghosh (2011) suggested that entropic stabilization may be largely responsible for the high melting temperature in hyperstable proteins and hints at residual structure or compactness of the denatured state in thermophiles. They showed that the gain in enthalpy upon folding is lower in thermophiles than in mesophiles, whereas the loss in entropy upon folding is higher in mesophiles than in thermophiles. The thermostable proteins have a slow unfolding rate, which helps to retain their near-native structures (Sawle and Ghosh 2011).

The thermostability of some enzymes is due to the presence of an extra repeat N-terminal domain (NTD) in the enzyme. For example, a novel thermostable SOD from *G. thermodenitrificans* NG80-2 exhibits maximum activity at 70 °C and high thermostability over a broad range of temperatures (20–80 °C). Unlike other reported SODs, this enzyme contains an extra repeat-containing NTD of 244 residues adjacent to the conserved functional SODA domain. It has been showed that the deletion of the NTD dramatically decreased its optimum active temperature (OAT) to 30 °C and also impaired its thermostability. Conversely, appending the NTD to a mesophilic counterpart from *B. subtilis* led to a moderately thermophilic enzyme (OAT changed from 30 to 55 °C) with improved heat resistance. The NTD also contributes to the stress resistance of host proteins without altering their metal ion specificity or oligomerization form except for a slight effect on their pH profile (Wang et al. 2014).

Metals such as zinc and calcium are often found in enzymes where they can stabilize a loop structure or hold secondary structures. The zinc ions, involved in the Zn-binding domain of thermoalkalophilic lipases from *G. thermocatenulatus* stabilized the structural arrangements of around 70 amino acids and the concerted movement of two lids, the 6- and 7-helices, during enzyme activation (Carrasco-Lopez et al. 2009). Ca ions restrict the conformational flexibility of certain helices and loops and bring about the stabilization of His residues through hydrogen bonding and thus lead to lipase thermostability (Sharma et al. 2013). Alpha-amylases and proteases isolated from various *Geobacillus* and *Anoxybacillus* spp. have been shown to contain Ca ions, which is enhancing the stability and activity of the enzymes at high temperatures (Eijsink et al. 2011; Chai et al. 2016).

5.4.4 Other Mechanisms for Thermostability

Large-scale genomic comparisons between thermophiles and mesophiles have shown that the genomes of thermophilic organisms have a higher guanine and cytosine (GC) content than mesophiles (Takami et al. 2004; Wang et al. 2015). It was hypothesized that a high GC content contributes to the thermostability of the genome and correlated with the optimum growth temperature of bacteria (Musto

et al. 2005; Musto et al. 2006). Additionally, tRNAs and rRNAs, the translational machinery of some thermophilic organisms, were reported to have high GC contents as well (Higgs and Ran 2008; Satapathy et al. 2010).

It has been observed that higher tRNA diversity usually occurs in thermophiles in comparison with non-thermophiles. Among psychrophiles, the total number of tRNA was found to be more than twofold higher than in the non-psychrophiles. The fact that growth temperature correlates with diversity and total amount of cellular tRNA (Satapathy et al. 2010) extends the list of molecular features undergoing adaptation due to growth temperature and supports the view that growth temperature acts as a strong selecting factor at the molecular level during evolution.

Small RNA (sRNA) has been shown to play important gene regulatory roles in the prokaryotes and can be involved in the adaptation at high temperatures. The sRNAs from *Geobacillus thermoleovorans* CCB_US3_UF5 strain, growing at 60–70 °C, were reverse transcribed to cDNA and sequenced. Sequencing data identified 83 putative sRNAs classified as antisense, intergenic region, untranslated region, or noncoding. Out of this total, 44 sRNA candidates were specific to growth at elevated temperature, suggesting that regulatory sRNA may play an important role in high-temperature adaptation in thermophilic bacteria (Tan and Alam 2010).

5.5 Biotechnological Potential of *Geobacillus* and *Anoxybacillus* Species

Members of the genera *Geobacillus* and *Anoxybacillus* can be used both in whole-cell applications and in biofuel and chemical production through engineered cells. One of the main advantages of using bacteria from these taxa is faster rate of growth, decreased contamination, and easier maintenance (Bertoldo and Antranikian 2002; Antranikian 2007). *Geobacillus* and *Anoxybacillus* species can be used as cell factories for multiple products, from gold nanoparticles using *Geobacillus* sp. strain ID17 (using NADH-dependent enzymes which convert Au^{3+} to elemental gold) (Correa-Llantén et al. 2013) to provision of thermostable enzymes (Zahoor et al. 2016; Sharma et al. 2013).

The members of these genera have a strong potential for application in bioremediation, especially with regard to degradation of aromatic compounds and removal of heavy metals. For example, 50 mg/L dried cells of *Geobacillus thermantarcticus* remove Cd^{2+}, Cu^{2+}, Co^{2+}, and Mn^{2+} up to 85.4%, 46.3%, 43.6%, and 65.1%, respectively, whereas *Anoxybacillus amylolyticus* removes 74.1%, 39.8%, 35.1%, and 36.6%, respectively, and *Anoxybacillus amylolyticus* removes the mentioned metal ions up to 74.1%, 39.8%, 35.1%, and 36.6%, respectively (Özdemir et al. 2013).

The ability of *Geobacillus* strains to metabolize aromatic compounds has been described by Feitkenhauer et al. (2003). They studied the kinetics of phenol degradation in continuous culture at 65 °C using *G. thermoleovorans*. Al-Jailawi et al. (2016) suggested that *A. rupiensis* strain Ir3 could be used as alternative to hydrodenitrogenation (HDN) for nitroaromatic compounds elimination (biotreatment) of crude oil and its derivatives. The quantitative analysis (HPLC) indicated that this

bacterium showed as much as 99.62% consumption of carbazole, 99.4% of ρ-nitrophenol, 97.73% of nitrobenzene, and 98.89% of naphthalene (Al-Jailawi et al. 2016).

Geobacillus and *Anoxybacillus* species demonstrate great versatility for adaptation and catalytic metabolism in a wide variety of environmental niches and are valuable sources of various thermostable enzymes. Thermophilic bacilli are of special interest as a source of novel thermostable enzymes and possess properties suitable for biotechnological and commercial use. There is, indeed, a considerable demand for a new generation of stable enzymes that are able to withstand severe conditions in industrial processes by replacing or supplementing traditional chemical processes. Their ability to conduct various reactions to higher process rates because of increase in substrate diffusion coefficient and reduced viscosity at higher temperatures makes them a preferred choice over mesophilic sources (Niehaus et al. 1999; Sharma et al. 2013).

Geobacillus and *Anoxybacillus* isolated from different hot springs show high potential as biocatalysts suitable for industrial biotechnology applications. The ability of these bacteria to produce a variety of extracellular enzymes, such as amylases, lipases, xylanases, proteases, esterases, and ureases, has ranked them among the most important enzyme producers (Bruins et al. 2001; Satyanarayana et al. 2012).

5.5.1 Amylases

Amylases are among the most important industrial enzymes and are of significance for their specific use in the starch conversion processes, having approximately 25% of the world enzyme market (Reddy et al. 2003). Amylolytic enzymes act on starch and related oligo- and polysaccharides, catalyzing the hydrolysis of internal α-1,4-glycosidic linkages in starch into low-molecular-weight products, such as glucose, maltose, and maltotriose units (Antranikian 2007).

A number of studies on starch-hydrolyzing enzymes based on the DNA sequence, structural analysis, and catalytic mechanism have led to the concept of one enzyme family: the alpha amylase. The amylolytic and related enzymes have been classified as glycoside hydrolases. They have been categorized as exoenzyme, endoenzyme, de-branching, and cyclodextrin-producing enzymes. The application of these enzymes has been established in a number of industrial processes such as food, fermentation, textiles, and paper industries (Antranikian 2007).

Amylolytic enzymes have been produced by a wide range of microorganisms. Heat-adapted amylases derived from the genus *Geobacillus* and *Anoxybacillus* have a big potential for commercial applications. α-Amylases from the members of genera *Geobacillus* and *Anoxybacillus* from the terrestrial hot springs are characterized by a high thermophilicity and stability (reacting between 30 and 120 °C) and activity within a wide range of pH values (from 5.5 to 13) (Table 5.4).

The α-amylases from thermophilic bacilli were purified and characterized with 42–97 kDa molecular weight (Table 5.4). Gurumurthy and Neelagund (2012) completed the molecular characterization of an extremely thermostable α-amylase

Table 5.4 Characteristics of thermostable α-amylases from *Geobacillus* and *Anoxybacillus* species

Microorganism	Source	Molecular weight (kDa)	pH optimal/ stability	Temperature optimal/ stability	Key observation	References
G. stearothermophilus	Mae'en hot springs in Jordan	–	7.0/5.5–13	55/25–75	Ca, Mn, Mg, and Cu slightly improve the enzymatic activity	Zakaria Al-Qodah (2006)
G. thermoleovorans	Hot spring of the Waimangu Volcanic Valley, New Zealand	–	7.0/ND	70/ND	Enzyme titer increased significantly in cane-molasses medium (60 U ml/1) as compared to that in the synthetic medium (26 U ml/1)	Maheswar Rao and Satyanarayana (2007)
G. thermoleovorans	Unkeshwar hot spring sediment, Nanded, India	42	7.5/5.5–9.0	68/65–90	The Km and Vmax values were 2.702 mg/ml and 7692.3 mmol, respectively. Ca, Cu, and Co ions increased activity	Rekadwad (2015)
Geobacillus sp.	Uttarakhand hot spring, Himalayan region, India	97	6.5/5.0–10	60/40–120	The values of *K*m and *V*max were 36 mg/ml and 222 μmol/mg/min, respectively	Dheeran et al. (2010)
Geobacillus sp.	Irde geothermal spring, Karnataka, India	43	8.0/5.0–11	90/45–95	Enzyme revealed about 55% α-helix, 5% β-strand, and 40% of unordered structure	Gurumurthy and Neelagund (2010, 2012)
Geobacillus sp.	Tengchong hot spring, China	67	5.6/ND	70/ND	Fe^{3+}, Cu^{2+}, EDTA inhibiting an enzyme activity	Wang et al. (2011b)
A. beppuensis	Tulsi Shyam hot spring reservoir, Gujarat, India	43	7.0/ND	80/50–90	*K*m and *V*max were 0.5 mg/ml and 3571.42 μmol/ml/m. Enzyme Ca ion independent and resistant to chemical	Kikani and Singh (2012)
A. thermarum	Hot mineral spring in Erzurum, Turkey	50	ND/5.5–10.5	70/20–90	The specific activity is 1203.7 U/mg. Enzyme activated by Ca, Cu, Ba, Co, and Zn ions	Baltas et al. (2016)

(continued)

Table 5.4 (continued)

Microorganism	Source	Molecular weight (kDa)	pH optimal/ stability	Temperature optimal/ stability	Key observation	References
Anoxybacillus sp.	Dargeçit hot spring, Turkey	85	7.0/ND	60/ND	*K*m and *V*max values were 0.102 µmol and 0.929 µmol/min, respectively	Acer et al. (2016)
Anoxybacillus sp.	Diyadin hot spring in Agri, Turkey	–	8.0/ND	60/ND	The maximum α-amylase production was secreted in the presence of 2% (w/v) soluble starch and casamino acid (14,310.6 U/mL)	Matpan Bekler and Güven (2014)
Anoxybacillus sp.	Hot spring in Malaysia	50	8.0/6.0–9.0	60/ND	The high immobilized enzyme activity retention (100%) and activity recovery (93%) achieved using ReliZyme HFA403/M	Kahar et al. (2016)
A. flavithermus	Omer hot springs, Afyonkarahisar, Turkey	60	7.0/6.0–8.0	55/35–70	Enzyme hydrolyzed soluble starch at 55 °C with Km: 0.005 mM and Vmax: 3.5 µmol/min	Fincan et al. (2013)
A. flavithermus	Karvachar hot spring, Nagorno-Karabakh	75	ND/5.5–8.5	ND/40–100	Amylase production started in early log phase and reached a maximum in late exponential phase with an activity of 205 U/ml	Panosyan et al. (2014)

ND not determined, *AN* accession number; – data not available

produced by a *Geobacillus* sp. for industrial applications. This α-amylase is considered as a novel enzyme due to its optimum activity at a very high temperature (90 °C) and at an alkaline condition (pH 8.0).

5.5.2 Lipases/Esterases

One of the important groups of biotechnologically relevant enzymes are lipases (EC 3.1.1.3 – triacylglycerol hydrolases), which have found large applications in food, diary, detergent, and pharmaceutical industries (Sharma et al. 2013; Gudiukaite et al. 2017). Lipases catalyze the hydrolysis of ester bonds of triacylglycerol at the interface between an insoluble substrate and water. In nonaqueous media these reactions are reversed due to a hydrophobic domain (lid), covering the active site of the lipase. The three-dimensional structures of lipases have a structural similarity with the α-/β-hydrolase family which contain terminal α-helices and a central β-sheet including the active Ser placed in a loop termed the catalytic elbow. Most α-/β--hydrolases contain a consensus sequence, Gly-X-Ser-X-Gly, around the active site serine, with a catalytic triad (Ser-Asp-His) (Ollis et al. 1992; Arpigny and Jaeger 1999; Gudiukaite et al. 2014).

Lipase-coding genes and activities have been reported in a wide range of microorganisms. However, lipases derived from thermophiles have privileges compared to the mesophilic lipases due to their unique attributes (Lotti and Alberghina 2007). Among the huge diversity of thermophilic bacteria, mainly bacilli have been reported as active thermostable lipase producers (Leow et al. 2004; Antranikian 2007; Sharma et al. 2013, Yang et al. 2013). A number of thermophilic bacilli species belonging to the genera *Geobacillus* and *Anoxybacillus* have been isolated from different geothermal springs and reported as thermostable lipase producers (Table 5.5).

The purified lipase from *Anoxybacillus* sp. isolated from the hot springs in Tăşnad (Romania) and Seferihisar Karakoc (Turkey) has a molecular weight of 25–26 kDa characterized by extremely high thermostability (25–90 °C) with optimum activity at 60–65 °C (Ay et al. 2011; Chis et al. 2013). Additional lipolytic enzymes from thermophilic bacilli were purified and characterized and possess molecular weights between 25 and 47 kDa (Ay et al. 2011; Balan et al. 2012; Chis et al. 2013; Mahadevan and Neelagund 2014).

The lipases purified from *G. thermodenitrificans* and *Geobacillus* sp. are characterized with 30–45 kDa molecular weight and act at the temperatures from 60 to 85 °C (Balan et al. 2012; Mahadevan and Neelagund 2014).

The lipases are stable at a wide range of pH values (5.0–11.0). The lipases from geobacilli mostly act at neutral pH, while the lipases from anoxybacilli are slightly alkaliphilic (Table 5.4).

Table 5.5 Thermostable lipase/esterase from *Geobacillus* and *Anoxybacillus* species and their characteristics

Microorganism	Source	Molecular weight (kDa)	pH optimal/ stability	Temperature optimal/ stability	Key observation	References
Geobacillus sp.	Tattapani hot springs, Himachal Pradesh, India	–	8.5/ND	60/ND	The *K*m and *V*max values of enzyme were 14 mM and 17.86μmol/ml/min, respectively. Enzyme activity increased in the presence of metal ions	Mehta et al. (2012)
G. thermodenitrificans	Hot spring in Labok, Kelantan, Malaysia	30	7.0/6.0–8.0	65/60–70	Enzyme showed elevated activity when pretreated with BaCl2, CaCl2, and KCl with 112%, 108%, and 106%, respectively. Lipase hydrolyzed tripalmitin (C16) and olive oil with optimal activity (100%) compared to other substrates	Balan et al. (2012), Yang et al. (2013)
Geobacillus sp.	Irde geothermal springs, Karnataka, India	47	8.0/8.0–12	70/60–85	The enzyme activity was promoted in the presence of Ca and Mg ions. The secondary structure of purified lipase contains 36% α-helix and 64% β-sheet	Mahadevan and Neelagund (2014)
Geobacillus sp.	Tatev hot spring, Armenia	–	6.0/4.0–8.0	65/45–75	The lipases belong to true lipases from family I and characterized with the presence of aspartic residues involved in Ca^{2+}-binding site	Shahinyan (2015), Shahinyan et al. (2017)
A. flavithermus	Hot spring in Tăşnad, Romania	25	6.5–8.0/3.0–9.0	60–65/25–80	Est/Lip is highly enantioselective, with preference for the (S)-enantiomer of substrates	Chis et al. (2013)
A. gonensis	Gonen hot springs, Turkey	–	7.5/5.5–9.5	60/50–70	Vmax and Km for the esterase activity of crude enzyme in the presence of p-nitrophenyl butyrate were 50 U/L and 0.125 mM, respectively. Ca^{2+} ion is cofactor	Colak et al. (2005)

Anoxybacillus sp.	Kuala Woh hot spring, Peninsular Malaysia	–	–	–	Lipase activity 0.56–2.62 U/ml	Olusesan et al. (2009)
Anoxybacillus sp.	Seferihisar Karakoc hot spring, Turkey	26	8.0/5.0–10.0	60/25–90	The enzyme exhibited a high level of activity with p-nitrophenyl butyrate with apparent Km, Vmax, and Kcat values of 0.348 ± 0.030 mM, 3725.8 U/mg, and 1500 ± 54.50/s, respectively	Ay et al. (2011)
Anoxybacillus sp.	Omer hot spring, Afyonkarahisar, Turkey	–	7.0/6.0–11	80/50–90	Co and Mg ions activated the enzyme by 188% and 149%, respectively	Ozdemir et al. (2015)
Anoxybacillus sp.	Karvachar hot spring, Nagorno-Karabakh	–	10.0/7.0–11.0	65/55–75	The lipases are close to the esterases and in primary structure display a Gly-Asp-Ser-(Leu) (GDSL) motif containing the active-site serine residue	Shahinyan (2015), Shahinyan et al. (2017)

ND not determined, – data not available

5.5.3 Proteases

Proteases are cleaving proteins into short peptides or free amino acids and are mainly divided into two major groups depending on their site of action: exopeptidases and endopeptidases (Sookkheo et al. 2002). Exopeptidases cleave the peptide bond proximal to the amino or carboxy termini of the substrate, whereas endopeptidases cleave the peptide bonds distant from the termini of the substrate. They can also be further divided into four groups based on the functional group present at the active site. These are serine, aspartic, cysteine, and metalloproteases (Rao et al. 1998).

Protease enzymes constitute one of the most important groups of industrial enzymes which are extensively used in the food, pharmaceutical, protein hydrolysis, detergent, cheese-making, brewing, photographic, baking, meat, and leather industries and inclusions in animal and human food as digestive aids (Seifzadeh et al. 2008; Synowiecki 2010).

There is a good correlation between growth temperature of the organism and the stability of its extracellular proteases. Thermophilic bacteria from hot springs are often good sources of thermostable proteases. *Geobacillus* and *Anoxybacillus* strains producing thermostable proteases are listed in Table 5.6.

The studied thermostable proteases of geobacilli and anoxybacilli isolated from the hot springs mostly exhibited optimum activity at a slightly alkaline pH (7–8) and are stable at the wide range of pH values (6–10). The thermostable alkaline proteases have been found to be the most appropriate enzyme in detergent industry, as the enzymes used in detergent formulations should have high activity and stability over a broad range of pH and temperature (Rao et al. 1998; Seifzadeh et al. 2008; Matpan Bekler et al. 2015).

5.5.4 Xylanases

Xylanase (EC 3.2.1.8) degrades the linear polysaccharide β-1,4-xylan into xylose, thus breaking down hemicellulose, one of the major components of plant cell walls. Biodegradation of xylan requires action of several enzymes, among which xylanase plays a key role. In the nature, the xylanase degrades the plant matter into usable nutrients and plays a major role in microbial thriving on plant sources. Microbial xylanases have large application in industry including the food, feed, fuel, textile, detergents, paper, and pulp industries and, also, in waste treatment (Kumar et al. 2013).

A number of thermophilic bacilli isolated from different terrestrial hot springs in Bulgaria (Derekova et al. 2008), Turkey (Kacagan et al. 2008; Inan et al. 2011, 2013), Japan (Sunna et al. 1997), India (Sharma et al. 2007), Pakistan (Zahoor et al. 2016), and the USA (Ellis and Magnuson 2012) were described as active xylanase producers. The purified and characterized xylanases of the species of *Geobacillus* and *Anoxybacillus* from the hot springs are listed in Table 5.7.

Table 5.6 Thermostable proteases from *Geobacillus* and *Anoxybacillus* species and their characteristics

Microorganism	Source	Molecular weight (kDa)	pH optimal/ stability	Temperature optimal/ stability	Key observation	References
Geobacillus sp.	Buranga hot spring, Uganda	97, 72, 50, 27, 22, 17, and 12	6.5/5.0–9.0	70/37–80	Proteases with 97 and 72 kDa molecular weight are heat-activated proteases, while the 12-kDa proteases are responsible for the observed protease activity at low temperature	Hawumba et al. (2002)
Geobacillus sp.	Tengchong hot spring, China	59.2	7.5/6.0–9.0	85/45–100	The activity of the protease is activated by Ca^{2+} and Mg^{2+} but inhibited partially by Ba^{2+}, Zn^{2+}, Pb^{2+}, Co^{2+}, Mn^{2+}, and Cu^{2+}	Zhu et al. (2007)
G. stearothermophilus	Hot spring in Chiang Mai, Thailand	36, 53, and 71	8.5, 7.5, and 7.0/6.0–10.0	70, 85, and 90/50–100	Classified as Zn^{2+} metalloproteases. The cleavage specificities of proteases S, N, and B on a 30-residue synthetic peptide from pro-BPN subtilisin were Tyr-Ile, Phe-Lys, and Gly-Phe, respectively	Sookkheo et al. (2002)
Anoxybacillus sp.	Köprü hot spring, Turkey	106	9.0/ND	50/50–60	The enzyme activity was increased in the presence of Ca^{2+}, Cu^{2+}, Tween 80, and Triton X-100	Matpan Bekler et al. (2015)
Anoxybacillus sp.	Hot spring Beppu city, Oita, Japan	57	6.0–8.0/5.5–10.0	70/50–90	The solubilization of sewage sludge by thermophilic protease is secreted	Nakamichi et al. (2010)

ND not determined

Table 5.7 Thermostable xylanases from *Geobacillus* and *Anoxybacillus* species and their characteristics

Microorganism	Source	Molecular weight (kDa)	pH optimal/ stability	Temperature optimal/ stability	Key observation	References
G. thermoleovorans	Hot spring in Kobe (Japan)	40 and 69	7.0/6.0–9.0	70–80/30–90	The crude xylanase complex is composed of two active bands	Sunna et al. (1997)
	Uttaranchal hot spring (India)	–	8.5/6.0–11.0	80/50–100	The treatment of the pulp with the xylanase (50 U g/1 dry pulp) was showed	Sharma et al. (2007)
A. gonensis *A. voinovskiensis* *A. kestanbolensis*	Dikili-Bergama Kaynarca hot spring, Camkoy Camur hot spring, Omerbeyli hot spring, Alangullu hot spring (Turkey)	–	9.0/ND	65–75/40–90	The alkaline active xylanases allow the direct enzymatic treatment of the alkaline pulp and avoid the cost incurring and time-consuming steps of pH readjustment	Inan et al. (2011)
A. kaynarcensis	Kaynarca hot Spring (Turkey)	100–150	7.0–9.0/ ND	65/ND	The presence of three xylanases in the cell supported by the zymogram of SDS-PAGE	Inan et al. (2013)
A. flavithermus	Alvord Basin hydrothermal system in Oregon (USA)	≈250	6.0–8.0/5.0–9.0	65/65–85	Pentameric protein complex consisting of protein subunits ranging from 25 to 75 kDa is suggested	Ellis and Magnuso (2012)
A. pushchinoensis	Diyadin hot springs (Turkey)	≈83	6.5/6.5–11.0	55/50–60	Vmax and Km determined at optimum temperature and were found to be 59.88 U/mg protein and 0.909 mg/ml, respectively	Kacagan et al. (2008)

ND not determined, – data not available

Thermophilic and alkaline active xylanases from *Anoxybacillus* species drive higher interest than other ones. The use of alkaline active xylanases allows direct enzymatic treatment of the alkaline pulp and avoids the cost of incurring and time-consuming steps of pH readjustment. Due to better solubility of xylan under alkaline conditions, alkaline active xylanase may also find other potential applications in addition to pulp bleaching (Inan et al. 2011, 2013).

5.5.5 Cellulases

Cellulose is the most abundant organic compound on Earth and has been extensively used as a substrate for the production of single-cell proteins, biofuels, and various other chemicals through microbial enzymatic degradation. The conversion of cellulosic biomass to fermentable sugars requires different types of cellulases, namely, β-1,4 endoglucanase (EC 3.4.1.4), β-1,4 exoglucanase (EC 3.2.1.91), and β-1,4 glucosidase (EC 3.2.1.21) (Sharma et al. 2015). Cellulose-degrading enzymes have various applications in starch processing, grain alcohol fermentation, deinking, drainage improvement, malting, and brewing. Thermostable cellulase is extensively used in the bio-stoning of denim fabrics and production of environment-friendly washing powders. In wine production cellulases are applied to obtain better fruit skin degradation, improved color extraction, easier must clarification, and better extraction (Kuhad et al. 2011).

Extracellular cellulases-producing anoxybacilli and geobacilli were mainly isolated from hot springs in Turkey (Cihan et al. 2014) and India (Sharma et al. 2015; Priya et al. 2016). The cellulases produced by *G. kaustophilus* PW11, *G. toebii* PW12, *G. thermoleovorans* PW13, *G. toebii* PS4, and *G. thermodenitrificans* IP_WH1strains isolated from Tattapani hot spring (India) were thermostable and exhibited activity even at 100 °C. Among the metal ions tested, Mn^{2+}, Co^{2+}, and Fe^{2+} significantly enhanced the cellulase activity, while Hg^{2+} (1 mM) strongly inhibited enzyme activity (Sharma et al. 2015; Priya et al. 2016). The activity of cellulase produced from *G. thermodenitrificans* IP_WH1 was higher (0.94 IU/ml at 60 °C) (Priya et al. 2016) than the activity of other thermophilic cellulases reported in the literature, such as *Bacillus* sp. with 0.14–0.37 IU/ml (Padilha et al. 2015) and *Bacillus* sp. SMIA-2 with 0.29 IU/ml (Ladeira et al. 2015) at 50 °C and pH 7.0.

Anoxybacillus gonensis isolated from Agri Diyadin hot spring (Turkey) produces a cellulase with approximately 40 kDa molecular weight, with highest activity at 50 °C and with an unusual broad optimum pH range (3–10) (Genc et al. 2015).

Cellulase-producing bacteria, such as *A. flavithermus* EHP1, *G. stearothermophilus* EHP2, and *G. thermodenitrificans* EHP3, have been isolated from Egyptian hot spring. The crude *A. flavithermus* EHP1 enzyme was produced at the end of the stationary phase and exhibited highest activity at 75 °C and pH 7.5 (Ibrahim and El-diwany 2007).

5.5.6 Exopolysaccharides

Exopolysaccharides (EPSs) are high-molecular-weight polymers composed of sugar residues. Bacteria produce diverse and multifunctional polysaccharides including intracellular, structural, and extracellular polysaccharides (exopolysaccharides). EPSs generally consist of polymers of monosaccharides and some non-carbohydrate substituents (such as acetate, pyruvate, succinate, and phosphate). EPSs play an important role for microbial cells, as they can form a protective layer for the cell against harsh external environments, serve as carbon and energy sources during starvation, mediate cell-cell interactions, facilitate the adherence of the cell to surface, and induce microbial aggregation or biofilm formation (Nwodo et al. 2012). Nichols et al. (2005), Junge et al. (2004), and Tourney and Ngwenya (2014) suggest also functions which include cryoprotection for growth at low temperatures, high-salinity tolerance with reference to sea ice microbial communities, and heavy metal precipitation on the cell surface.

The various properties of microbial EPS have found large application in the industry. EPS like xanthan and gellan are already utilized in the food industry as gelling agents and thickeners for salad dressings, desserts, sauces, syrups, and ice cream (Kornmann et al. 2003). New areas for the application of microbial polysaccharides include improving the efficiency of liquid herbicides and insecticides; stabilization of emulsified pharmaceutical and cosmetic creams (Moonmangmee et al. 2002; Sutherland 1999), as thickeners and stabilizers in shampoos, toothpaste, and makeup; and solidifier of microbiological and plant tissue culture media. In recent years there has been an increasing interest in their biological activities, like antitumor, antiviral, immunostimulatory (Arena et al. 2006; Weiner et al. 1995), and anti-inflammatory effects (De Stefano et al. 2007).

EPSs from thermophilic bacteria offer numerous applications in various fields of industry, as the thermophiles provide more suitable processes for polymer production with decreased viscosity at high temperature. Extremophiles offer a great diversity in chemical and physical properties of their EPS compared to anywhere else in the biosphere (Guezennec 2002). Additionally, EPSs synthesized by thermophilic bacteria are likely to keep their structural properties at high temperature, which is a desired feature of the polymer solution (Radchenkova et al. 2013).

EPSs from geobacilli and anoxybacilli isolated from different geothermal springs are promising for their use in the industry. One gram of EPS from *Anoxybacillus* sp. R4-33 isolated from a hot spring in China absorbed 1.9783 mg Zn(II) and 1.4095 mg Cd(II) at pH 6.0 (Zhao et al. 2014). This EPS was a heteropolysaccharide, composed of D-mannose and D-glucose as its principal monosaccharide components in the relative proportions 1:0.45 (Table 5.8). Production of thermostable EPS was also reported for *G. tepidamans* (Kambourova et al. 2009), *G. thermodenitrificans* (Panosyan 2017, Panosyan et al. 2014), *G. toebii*, and *A. kestanbolensis* (Radchenkova et al. 2013; Panosyan 2017).

Table 5.8 Thermostable EPS from *Geobacillus* and *Anoxybacillus* species and their characteristics

Microorganism	Isolation source	Carbon Source	EPS yield (mg l^{-1})	EPS molecular weight (kDa), chemical composition (relative ratio)	References
G. toebii	Rupi hot spring, Bulgaria	Sucrose	50	ND	Radchenkova et al. (2013)
G. tepidamans V264	Velingrad hot spring, Bulgaria	Maltose	111.4	1000, Glc/Gal/Fuc/Fru (1/0.07/0.04/0.02)	Kambourova et al. (2009) and Coorevits et al. (2011)
G. thermodenitrificans ArzA-6	Arzakan geothermal spring, Armenia	Fructose/glucose	76	500, Man/Gal/Ara/Fru/Glc (1/0.13/0.1/0.06/0.05)	Panosyan (2017) and Panosyan et al. (2014)
G. toebii ArzA-8			80	600, Man/Gal/Glc/Ara (1/0.5/0.2/0.05)	
A. kestanbolensis 415	Mizinka hot spring, Bulgaria	Sucrose	25.3	ND	Radchenkova et al. (2013)
Anoxybacillus sp. *R4-33*	Radioactive radon hot spring, China	Glucose	1083	EPSII, 1000, Man/Glc (1/0.45)	Zhao et al. (2014)

ND not determined

5.6 Conclusion

Isolation and study of thermophilic bacilli from terrestrial geothermal springs are important for understanding of the diversity of thermophilic microbes and exploring their biotechnological potency. Many new thermophilic microbes belonging to the genera *Anoxybacillus* and *Geobacillus* have been isolated from different terrestrial geothermal springs worldwide, identified, and evaluated taking into account their biotechnological potency. *Anoxybacillus* is a relatively new genus compared to the well-studied *Geobacillus*. Most of the reported data has revealed that the members of both genera produce interesting enzymes that are thermostable and tolerant to alkaline conditions. Some of the studied enzymes were discovered through partnerships with industry. The interest in heat-adapted industrial enzymes is expected to increase. The present work, therefore, extends the previous sphere of information regarding the thermophilic bacilli diversity of terrestrial geothermal springs worldwide and their biotechnological applications and potency.

Acknowledgments This work was supported by grants from the EURASIA Programme of the Norwegian Center for International Cooperation in Education (CPEA-2011/10081, CPEA-LT-2016/10095) and partially supported by the RA MES State Committee of Science, in the frames of the research project no. 15T-1F399, and the Armenian National Science and Education Fund based in New York, USA, to HP (ANSEF-NS-microbio 2493, 3362, and 4676).

References

Acer Ö, Bekler FM, Pirinççioğlu H, Güven RG, Güven K (2016) Purification and Characterization of Thermostable and Detergent-Stable α-Amylase from *Anoxybacillus* sp. AH1. Food Technol Biotechnol 54(1):70–77

Acer Ö, Pırınççiğlu H, Bekler Fatma M, Gül-Güven R (2015) *Anoxybacillus* sp. AH1, an α-amylase-producing thermophilic bacterium isolated from Dargeçit hot spring. Biologia Sect Cell Mol Biol 70(7):853–862

Agüloğlu Fincan S, Enez B, Özdemir S, Matpan Bekler F (2014) Purification and characterization of thermostable α-amylase from thermophilic *Anoxybacillus flavithermus*. Carbohydr Polym 102(Supplement C):144–150

Ahmad S, Scopes RK, Rees GN, Patel BKC (2000) *Saccharococcus caldoxylosilyticus* sp. nov., an obligately thermophilic, xylose-utilizing, endospore-forming bacterium. Int J Syst Evol Microbiol 50:517–523

Al-Batayneh KM, Jacob JH, Hussein EI (2011) Isolation and molecular identification of new thermophilic bacterial strains of *Geobacillus pallidus* and *Anoxibacillus flavithermus*. Int J Integr Biol 11(1):39–43

Al-Jailawi MH, Mahdi MS, Fadhil AMA (2016) Investigation the Consumption of Aromatic Hydrocarbons by *Anoxybacillus rupeinsis* Ir3 (JQ912241) using FTIR and HPLC Analyses. Br Biotechnol J 10(2):1–12 Article no. BBJ.18203

Al-Qodah Z (2006) Production and characterization of thermostable alpha-amylase by thermophilic *Geobacillus stearothermophilus*. Biotechnol J 1(7-8):850–857

Antranikian G (2007) Industrial relevance of thermophiles and their enzymes. In: Thermophiles. CRC Press, pp 113–160

Arena A, Maugeri TL, Pavone B, Iannello D, Gugliandolo C, Bisignano G (2006) Antiviral and immunoregulatory effect of a novel exopolysaccharide from a marine thermotolerant *Bacillus licheniformis*. Int Immunopharmacol 6(1):8–13

Arpigny JL, Jaeger KE (1999) Bacterial lipolytic enzymes: classification and properties. Biochem J 343(Pt 1):177–183

Arya M, Joshi GK, Gupta AK, Kumar A, Raturi A (2015) Isolation and characterization of thermophilic bacterial strains from Soldhar (Tapovan) hot spring in Central Himalayan Region, India. Ann Microbiol 65(3):1457–1464. https://doi.org/10.1007/s13213-014-0984-y

Ash C, Farrow JAE, Wallbanks S, Collins MD (1991) Phylogenetic heterogeneity of the genus *Bacillus* revealed by comparative analysis of small subunit ribosomal RNA sequences. Lett Appl Microbiol 13:202–206

Atanassova M, Derekova A, Mandeva R, Sjoholm C, Kambourova M (2008) *Anoxybacillus bogrovensis* sp. nov., a novel thermophilic bacterium isolated from a hot spring in Dolni Bogrov, Bulgaria. Int J Syst Evol Microbiol 58(Pt 10):2359–2362

Ay F, Karaoglu H, Inan K, Canakci S, Belduz AO (2011) Cloning, purification and characterization of a thermostable carboxylesterase from *Anoxybacillus* sp. PDF1. Protein Expr Purif 80(1):74–79

Balan A, Ibrahim D, Abdul Rahim R, Ahmad Rashid FA (2012) Purification and characterization of a thermostable lipase from *Geobacillus thermodenitrificans* IBRL-nra. Enzyme Res 2012:987523. https://doi.org/10.1155/2012/987523

Baltas N, Dincer B, Ekinci AP, Kolayli S, Adiguzel A (2016) Purification and characterization of extracellular α-amylase from a thermophilic *Anoxybacillus thermarum* A4 strain, Braz. arch. biol. technol. Eng Technol Tech 59:e16160346. https://doi.org/10.1590/1678-4324-2016160346

Banat IM, Marchant R, Rahman TJ (2004) *Geobacillus debilis* sp. nov., a novel obligately thermophilic bacterium isolated from a cool soil environment, and reassignment of *Bacillus pallidus* to *Geobacillus pallidus* comb. nov. Int J Syst Evol Microbiol 54:2197–2201

Belduz AO, Canakci S, Chan K-G, Kahar UM, Chan CS, Yaakop AS, Goh KM (2015) Genome sequence of *Anoxybacillus ayderensis* AB04(T) isolated from the Ayder hot spring in Turkey. Stand Genomic Sci 10:70. https://doi.org/10.1186/s40793-015-0065-2

Belduz AO, Dulger S, Demirbag Z (2003) *Anoxybacillus gonensis* sp. nov., a moderately thermophilic, xylose-utilizing, endospore-forming bacterium. Int J Syst Evol Microbiol 53(Pt 5):1315–1320

Bergey DH, Whitman WB, De Vos P, Garrity GM and Jones D (2009) Bergey's manual of systematic bacteriology. 3, New York: Springer.: https://doi.org/10.1002/9781118960608.gbm00529

Bertoldo C, Antranikian G (2002) Starch-hydrolyzing enzymes from thermophilic archaea and bacteria. Curr Opin Chem Biol 6(2):151–160. https://doi.org/10.1016/S1367-5931(02)00311-3

Bruins ME, Janssen AE, Boom RM (2001) Thermozymes and their applications: a review of recent literature and patents. Appl Biochem Biotechnol 90(2):155–186

Brumm P, Land ML, Hauser LJ, Jeffries CD, Chang Y-J, Mead DA (2015) Complete Genome Sequence of *Geobacillus* strain Y4.1MC1, a Novel CO-Utilizing *Geobacillus thermoglucosidasius* Strain Isolated from Bath Hot Spring in Yellowstone National Park. BioEnergy Res 8(3):1039–1045

Bryanskaya AV, Rozanov AS, Slynko NM, Shekhovtsov SV, Peltek SE (2015) *Geobacillus icigianus* sp. nov., a thermophilic bacterium isolated from a hot spring. Int J Syst Evol Microbiol 65(Pt 3):864–869

Caccamo D, Gugliandolo C, Stackebrandt E, Maugeri TL (2000) *Bacillus vulcani* sp. nov., a novel thermophilic species isolated from a shallow marine hydrothermal vent. Int J Syst Evol Microbiol 50:2009–2012

Canakci S, Inan K, Kacagan M, Belduz AO (2007) Evaluation of arabinofuranosidase and xylanase activities of *Geobacillus* spp. isolated from some hot springs in Turkey. J Microbiol Biotechnol 17(8):1262–1270

Carrasco-Lopez C, Godoy C, de Las Rivas B, Fernandez-Lorente G, Palomo JM, Guisan JM, Fernandez-Lafuente R, Martinez-Ripoll M and Hermoso JA (2009) Activation of bacterial thermoalkalophilic lipases is spurred by dramatic structural rearrangements, J Biol Chem 7. 4365-4372

Chai KP, Othman NF, Teh AH, Ho KL, Chan KG, Shamsir MS, Goh KM, Ng CL (2016) Crystal structure of *Anoxybacillus* alpha-amylase provides insights into maltose binding of a new glycosyl hydrolase subclass. Sci Rep 6:23126. https://doi.org/10.1038/srep23126

Chai YY, Kahar UM, Md Salleh M, Md Illias R, Goh KM (2012) Isolation and characterization of pullulan-degrading *Anoxybacillus* species isolated from Malaysian hot springs. Environ Technol 33(10-12):1231–1128

Chakravorty D, Patra S (2013) Attaining Extremophiles and Extremolytes: Methodologies and Limitations. In: Singh OV (ed) Extremophiles: sustainable resources and biotechnological implications, 1st edn. Wiley, Hoboken, pp 29–74

Chang YW, Sun YJ, Wang C, Hsiao CD (2008) Crystal structures of the 70-kDa heat shock proteins in domain disjoining conformation. J Biol Chem 283(22):15502–15511

Chatziefthimiou AD, Crespo-Medina M, Wang Y, Vetriani C, Barkay T (2007) The isolation and initial characterization of mercury resistant chemolithotrophic thermophilic bacteria from mercury rich geothermal springs. Extremophiles 11(3):469–479. https://doi.org/10.1007/s00792-007-0065-2

Che Ibrahim MA, Ahmad WA (2017) Growth optimization of a thermophilic strain *Geobacillus caldoxylosilyticus* utm6 isolated from selayang hot spring. eProc Chem 2(1):119–123

Chis L, Hriscu M, Bica A, Tosa M, Nagy G, Rona G, Vertessy BG, Dan Irimie F (2013) Molecular cloning and characterization of a thermostable esterase/lipase produced by a novel *Anoxybacillus flavithermus* strain. J Gen Appl Microbiol 59(2):119–134

Cihan AC, Cokmus C, Koc M, Ozcan B (2014) *Anoxybacillus calidus* sp. nov., a thermophilic bacterium isolated from soil near a thermal power plant. Int J Syst Evol Microbiol 64(Pt 1):211–219

Cihan AC, Ozcan B, Cokmus C (2011) *Anoxybacillus salavatliensis* sp. nov., an alpha-glucosidase producing, thermophilic bacterium isolated from Salavatli, Turkey. J Basic Microbiol 51(2):136–146

Colak A, Sisik D, Saglam N, Guner S, Canakci S, Belduz AO (2005) Characterization of a thermoalkalophilic esterase from a novel thermophilic bacterium, *Anoxybacillus gonensis* G2. Bioresour Technol 96(5):625–631

Coorevits A, Dinsdale AE, Halket G, Lebbe L, De Vos P, Van Landschoot A, Logan NA (2012) Taxonomic revision of the genus *Geobacillus*: emendation of *Geobacillus, G. stearothermophilus, G. jurassicus, G. toebii, G. thermodenitrificans* and *G. thermoglucosidans* (nom. corrig., formerly 'thermoglucosidasius'); transfer of *Bacillus thermantarcticus* to the genus as *G. thermantarcticus* comb. nov.; proposal of *Caldibacillus debilis* gen. nov., comb. nov.; transfer of *G. tepidamans* to *Anoxybacillus* as *A. tepidamans* comb. nov.; and proposal of *Anoxybacillus caldiproteolyticus* sp. nov. Int J Syst Evol Microbiol 62(Pt 7):1470–1485

Correa-Llantén DN, Muñoz-Ibacache SA, Castro ME, Muñoz PA, Blamey JM (2013) Gold nanoparticles synthesized by *Geobacillus* sp. strain ID17 a thermophilic bacterium isolated from Deception Island, Antarctica. BMC Microb Cell Fact 12(75):1–6. https://doi.org/10.1186/1475-2859-12-75

Cortez Y, Paul S, Villena G, Gutiérrez-Correa M (2016) Isolation and characterization of cellulase producing bacterial strains from an Amazonian geothermal spring in Peru. Br Microbiol Res J 15(5):1–8

Dai J, Liu Y, Lei Y, Gao Y, Han F, Xiao Y, Peng H (2011) A new subspecies of *Anoxybacillus flavithermus* ssp. *yunnanensis* ssp. nov. with very high ethanol tolerance. FEMS Microbiol Lett 320(1):72–78

Daupan SMMV, Rivera WL (2015) Isolation and characterization of culturable thermophilic bacteria from hot springs in Benguet, Philippines. Int J Philipp Sci Technol 8(2):14–18

De Clerck E, Rodríguez-Díaz M, Vanhoutte T, Heyrman J, Logan NA, De Vos P (2004) *Anoxybacillus contaminans* sp. nov., and *Bacillus gelatini* sp. nov., isolated from contaminated gelatin batches. Int J Syst Evol Microbiol 54:941–946

De Maayer P, Brumm PJ, Mead DA, Cowan DA (2014) Comparative analysis of the *Geobacillus hemicellulose* utilization locus reveals a highly variable target for improved hemicellulolysis. BMC Genomics 15(1):836. https://doi.org/10.1186/1471-2164-15-836

Deep K, Poddar A, Whitman WB, Das SK (2016) Draft Genome Sequence of *Anoxybacillus suryakundensis* Strain JS1T (DSM 27374T) Isolated from a Hot Spring in Jharkhand, India. Genome Announc 4(4):pii: e00824-16. https://doi.org/10.1128/genomeA.00824-16

Derekova A, Mandeva R, Kambourova M (2008) Phylogenetic diversity of thermophilic carbohydrate degrading bacilli from Bulgarian hot springs. World J Microbiol Biotechnol 24(9):1697–1702

Derekova A, Sjoholm C, Mandeva R, Kambourova M (2007) *Anoxybacillus rupiensis* sp. Nov., a novel thermophilic bacterium isolated from Rupi basin (Bulgaria). Extremophiles 11(4):577–583

Derekova A, Sjoholm C, Mandeva R, Michailova L, Kambourova M (2006) Biosynthesis of a thermostable gellan lyase by newly isolated and characterized strain of *Geobacillus stearothermophilus* 98. Extremophiles 10(4):321–326

De Stefano D, Tommonaro G, Simeon V, Poli A, Nicolaus B, Carnuccio R (2007) A polysaccharide from tomato (*Lycopersicon esculentum*) peels affects NF-jB activation in LPS-stimulated J774 macrophages. J Nat Prod 70:1636–1639

Dheeran P, Kumar S, Jaiswal YK, Adhikari DK (2010) Characterization of hyperthermostable alpha-amylase from *Geobacillus* sp. IIPTN. Appl Microbiol Biotechnol 86(6):1857–1866

Donk PJ (1920) A Highly Resistant Thermophilic Organism. Journal of Bacteriology 5(4):373–374
Dulger S, Demirbag Z, Belduz AO (2004) *Anoxybacillus ayderensis* sp. nov. and *Anoxybacillus kestanbolensis* sp. nov. Int J Syst Evol Microbiol 54(Pt 5):1499–1503
Eijsink VG, Matthews BW, Vriend G (2011) The role of calcium ions in the stability and instability of a thermolysin-like protease. Protein Sci 20(8):1346–1355
Ellis JT, Magnuson TS (2012) Thermostable and Alkalistable Xylanases Produced by the Thermophilic Bacterium *Anoxybacillus flavithermus* TWXYL3. ISRN Microbiology 12:ID 517524. https://doi.org/10.5402/2012/517524
England JL, Shakhnovich BE, Shakhnovich EI (2003) Natural selection of more designable folds: a mechanism for thermophilic adaptation. Proc Natl Acad Sci USA 100(15):8727–8731
Feitkenhauer H, Muller R, Markl H (2003) Degradation of polycyclic aromatic hydrocarbons and long chain alkanes at 60–70 degrees C by *Thermus* and *Bacillus* spp [corrected]. Biodegradation 14(6):367–372
Filippidou S, Jaussi M, Junier T, Wunderlin T, Jeanneret N, Palmieri F, Palmieri I, Roussel-Delif L, Vieth-Hillebrand A, Vetter A, Chain PS, Regenspurg S, Junier P (2016) *Anoxybacillus geothermalis* sp. nov., a facultatively anaerobic, endospore-forming bacterium isolated from mineral deposits in a geothermal station. Int J Syst Evol Microbiol 66(8):2944–2951
Fortina MG, Mora D, Schumann P, Parini C, Manachini PL, Stackebrandt E (2001) Reclassification of *Saccharococcus caldoxylosilyticus* as *Geobacillus caldoxylosilyticus* (Ahmad et al. 2000) comb. nov. Int J Syst Evol Microbiol 51:2063–2071
Genc B, Nadaroglu H, Adiguzel A, Baltaci O (2015) Purification and characterization of an extracellular cellulase from *Anoxybacillus gonensis* O9 isolated from geothermal area in Turkey. J Environ Biol 36(6):1319–1324
Goh KM, Gan HM, Chan K-G, Chan GF, Shahar S, Chong CS, Kahar UM, Chai KP (2014) Analysis of *Anoxybacillus* genomes from the aspects of lifestyle adaptations, prophage diversity, and carbohydrate metabolism. PLOS One 9(3):e90549
Graham R, Ternan N, McMullan G (2005) Proteomic investigation of *Geobacillus thermoleovorans* T80. In: Proceedings of international symposium on extremophiles and their applications, pp 97–101
Gudiukaite R, Sadauskas M, Gegeckas A, Malunavicius V, Citavicius D (2017) Construction of a novel lipolytic fusion biocatalyst GDEst-lip for industrial application. J Ind Microbiol Biotechnol 44(6):799–815
Gudiukaite R, Gegeckas A, Kazlauskas CD (2014) Influence of N- and C-terminal regions on activity, expression, characteristics and structure of lipase from *Geobacillus* sp. 95. Extremophiles 18:131–145
Guezennec J (2002) Deep-sea hydrothermal vents: A new source of innovative bacterial exopolysaccharides of biotechnological interest. J Ind Microbiol Biotechnol 29:204–208
Gul-Guven R, Guven K, Poli A, Nicolaus B (2008) *Anoxybacillus kamchatkensis* subsp. *asaccharedens* subsp. nov., a thermophilic bacterium isolated from a hot spring in Batman. J Gen Appl Microbiol 54(6):327–334
Gurumurthy DM, Neelagund SE (2010) *Geobacillus* sp. Iso 5, a novel amylase-producing thermophile from thermal springs in Konkan region of Southern India. J Earth Sci 21(1):319–322
Gurumurthy DM, Neelagund SE (2012) Molecular characterization of industrially viable extreme thermostable novel α-amylase of *Geobacillus* sp. Iso5 isolated from geothermal spring. J Pure Appl Microbiol 6(4):1–15
Hawumba JF, Theron J, Brozel VS (2002) Thermophilic protease-producing *Geobacillus* from Buranga hot springs in Western Uganda. Curr Microbiol 45(2):144–150
Heinen W, Lauwers AM, Mulders JW (1982) *Bacillus flavothermus*, a newly isolated facultative thermophile. Antonie Van Leeuwenhoek 48(3):265–272
Higgs PG, Ran W (2008) Coevolution of codon usage and tRNA genes leads to alternative stable states of biased codon usage. Mol Biol Evol 25(11):2279–2291
Hovhannisyan P, Panosyan H, Turabyan A, Trchounian A (2016) Termostable amylase producing bacilli isolated from Armenian geothermal springs. Biol J Armenia 68(Special Issue):68–73

Hussein AH, Lisowska BK, Leak DJ (2015) The genus *Geobacillus* and their biotechnological potential. Adv Appl Microbiol 92:1–48

Ibrahim ASS, El-diwany AI (2007) Isolation and identification of new cellulases producing thermophilic bacteria from an Egyptian hot spring and some properties of the crude enzyme. Aust J Basic Appl Sci 1(4):473–478

Inan K, Belduz AO, Canakci S (2013) *Anoxybacillus kaynarcensis* sp. nov., a moderately thermophilic, xylanase producing bacterium. J Basic Microbiol 53(5):410–419

Inan K, Canakci S, Belduz A (2011) Isolation and characterization of xylanolytic new strains of A*noxybacillus* from some hot springs in Turkey. Turk J Biol 35:529–542

Jorquera MA, Gabler S, Inostroza NG, Acuna JJ, Campos MA, Menezes-Blackburn D, Greiner R (2018) Screening and characterization of phytases from bacteria isolated from Chilean hydrothermal environments. Microb Ecol 75(2):387–399

Jugran J, Rawat N, Joshi G (2015) Amylase production by *Geobacillus* sp. GJA1 isolated from a hot spring in Uttarakhand. ENVIS Himalayan Ecol 23:120–125

Junge K, Eicken H, Deming JW (2004) Bacterial Activity at −2 to −20 degrees C in Arctic wintertime sea ice. Appl Environ Microbiol 70(1):550–557

Kacagan M, Canakci S, Sandalli C, Inan K, Colak DN, Belduz AO (2008) Characterization of a xylanase from a thermophilic strain of *Anoxybacillus pushchinoensis* A8. Biologia 63(5):599–606

Kahar UM, Sani MH, Chan KG, Goh KM (2016) Immobilization of alpha-Amylase from *Anoxybacillus* sp. SK3-4 on ReliZyme and Immobead Supports. Molecules 21(9):1196. https://doi.org/10.3390/molecules21091196

Kambourova M, Mandeva R, Dimova D, Poli A, Nicolaus B, Tommonaro G (2009) Production and characterization of a microbial glucan, synthesized by *Geobacillus tepidamans* V264 isolated from Bulgarian hot spring. Carbohydrate Polymers 77(2):338–343

Kevbrin VV, Zengler K, Lysenko AM, Wiegel J (2005) *Anoxybacillus kamchatkensis* sp. nov., a novel thermophilic facultative aerobic bacterium with a broad pH optimum from the Geyser valley, Kamchatka. Extremophiles 9(5):391–398

Kikani BA, Singh SP (2012) The stability and thermodynamic parameters of a very thermostable and calcium-independent α-amylase from a newly isolated bacterium, *Anoxybacillus beppuensis* TSSC-1. Process Biochem 47(12):1791–1798

Khalil A, Sivakumar N, Qarawi S (2015) Genome Sequence of *Anoxybacillus flavithermus* Strain AK1, a Thermophile Isolated from a Hot Spring in Saudi Arabia. Genome Announc 3(3):e00604-15

Kornmann H, Duboc P, Marison I, von Stockar U (2003) Influence of nutritional factors on the nature, yield, and composition of exopolysaccharides produced by *Gluconacetobacter xylinus* I-2281. Appl Environ Microbiol 69(10):6091–6098

Kuisiene N, Raugalas J, Chitavichius D (2004) *Geobacillus lituanicus* sp. nov. Int J Syst Evol Microbiol 54:1991–1995

Kuhad RC, Gupta R, Singh A (2011) Microbial Cellulases and Their Industrial Applications. Enzyme Res 2011(280696). https://doi.org/10.4061/2011/280696

Kumar V, Verma D, Archana A, Satyanarayana T (2013) Thermostable Bacterial Xylanases. In: Satyanarayana T, Littlechild J, Kawarabayasi Y (eds) Thermophilic microbes in environmental and industrial biotechnology: biotechnology of thermophiles. Springer, Dordrecht, pp 813–857

Ladeira SA, Cruz E, Delatorre AB, Barbosa JB, Martins MLL (2015) Cellulase production by thermophilic *Bacillus* sp. SMIA-2 and its detergent compatibility. Electron J Biotechnol 18(2):110–115. https://doi.org/10.1016/j.ejbt.2014.12.008

Lee SJ, Lee YJ, Ryu N, Park S, Jeong H, Kim BC, Lee DW, Lee HS (2012) Draft genome sequence of the thermophilic bacterium *Anoxybacillus kamchatkensis* G10. J Bacteriol 194(23):6684–6685

Leow TC, Rahman RN, Basri M, Salleh AB (2004) High level expression of thermostable lipase from *Geobacillus* sp. strain T1. Biosci Biotechnol Biochem 68(1):96–103

Lim YL, Chan KG, Ee R, Belduz AO, Canakci S, Kahar UM, Yaakop AS, Goh KM (2015) Complete genome of the potential thermozyme producer *Anoxybacillus gonensis* G2(T) isolated from the Gonen hot springs in Turkey. J Biotechnol 212:65–66. https://doi.org/10.1016/j.jbiotec.2015.08.007

Liu Y, Li R, Wang J, Zhang X, Jia R, Gao Y, Peng H (2017) Increased enzymatic hydrolysis of sugarcane bagasse by a novel glucose- and xylose-stimulated β-glucosidase from *Anoxybacillus flavithermus* subsp. *yunnanensis* E13(T). BMC Biochem 18:art4, 1–8. https://doi.org/10.1186/s12858-017-0079-z

Llarch A, Logan NA, Castellvi J, Prieto MJ, Guinea J (1997) Isolation and characterization of thermophilic *Bacillus* spp. from geothermal environments on Deception Island, South Shetland Archipelago. Microb Ecol 34(1):58–65

Lobry JR, Chessel D (2003) Internal correspondence analysis of codon and amino-acid usage in thermophilic bacteria. J Appl Genet 44(2):235–261

Lotti M, Alberghina L (2007) Lipases: molecular structure and function. In: Polaina J, MacCabe AP (eds) Industrial enzymes: structure, function and applications, vol 16. Springer, Dordrecht, pp 264–282

Mahadevan GD, Neelagund SE (2014) Thermostable lipase from *Geobacillus* sp. Iso5: bioseparation, characterization and native structural studies. J Basic Microbiol 54(5):386–396. https://doi.org/10.1002/jobm.201200656

Mahadevan GD, Neelagund SE (2010) *Geobacillus* sp. Iso 5, a novel amylase producing thermophile from konkan region thermal springs in southern India. J Earth Sci 21:318–322

Matpan Bekler F, Acer O, Guven K (2015) Production and purification of novel thermostable alkaline protease from *Anoxybacillus* sp. KP1. Cell Mol Biol (Noisy-le-grand) 61(4):113–120

Matpan Bekler F, Güven K (2014) Isolation and production of thermostable α-amylase from thermophilic *Anoxybacillus* sp. KP1 from Diyadin hot spring in Ağri, Turkey. Biologia 69(4):419–427

Mehta D, Satyanarayana T (2013a) Biochemical and molecular characterization of recombinant acidic and thermostable raw-starch hydrolysing α-amylase from an extreme thermophile *Geobacillus thermoleovorans*. J Mol Catal B: Enzym 85–86(0):229–238

Mehta D, Satyanarayana T (2013b) Diversity of hot environments and thermophilic microbes'. In: Satyanarayana T, Littlechild J, Kawarabayasi Y (eds) Thermophilic microbes in environmental and industrial biotechnology: biotechnology of thermophiles. Springer, Dordrecht, pp 3–60

Mehta A, Kumar R, Gupta R (2012) Isolation of lipase producing thermophilic bacteria: optimization of production and reaction conditions for lipase from *Geobacillus* sp. Acta Microbiol Immunol Hung 59(4):435–450

Miñana-Galbis D, DL P´ n, JG L´n, Manresa A, OliartRos RM (2010) Reclassification of *Geobacillus pallidus* (Scholz et al. 1988) Banat et al. 2004 as *Aeribacillus pallidus* gen. nov., comb. nov. Int J Syst Evol Microbiol 60:1600–1604

Mollania N, Khajeh K, Hosseinkhani S, Dabirmanesh B (2010) Purification and characterization of a thermostable phytate resistant alpha-amylase from *Geobacillus* sp. LH8. Int J Biol Macromol 46(1):27–36

Moonmangmee S, Toyama H, Adachi O, Teerakool G, Lotong N, Matsushita K (2002) Purification and characterization of a novel polysaccharide involved in the pellicle produced by a thermotolerant *Acetobacter* strain. Biosci Biotechnol Biochem 66:777–783

Muhd Sakaff MK, Abdul Rahman AY, Saito JA, Hou S, Alam M (2012) Complete genome sequence of the thermophilic bacterium *Geobacillus thermoleovorans* CCB_US3_UF5. J Bacteriol 194(5):1239

Musto H, Naya H, Zavala A, Romero H, Alvarez-Valin F, Bernardi G (2005) The correlation between genomic G+C and optimal growth temperature of prokaryotes is robust: a reply to Marashi and Ghalanbor. Biochem Biophys Res Commun 330(2):357–360

Musto H, Naya H, Zavala A, Romero H, Alvarez-Valin F, Bernardi G (2006) Genomic GC level, optimal growth temperature, and genome size in prokaryotes. Biochem Biophys Res Commun 347(1):1–3

Nakamichi T, Nakashima T, Fujisaki H, Nobue T, Muramatsu T, Takahashi Y, Ishibashi Y (2010) Characteristics of *Anoxybacillus* sp. MU3 isolated from a hot spring and its application to the hyper thermal solubilization of sewage sludge. Environ Eng Sci 27(12):993–999

Namsaraev ZB, Babasanova OB, Dunaevsky YE, Akimov VN, Barkhutova DD, Gorlenko VM, Namsaraev BB (2010) *Anoxybacillus mongoliensis* sp. nov., a novel thermophilic protein-

ase producing bacterium isolated from alkaline hot spring, central Mongolia. Mikrobiologiia 79(4):516–523

Narayan VV, Hatha MA, Morgan HW, Rao D (2008) Isolation and characterization of aerobic thermophilic bacteria from the savusavu hot springs in fiji. Microbes Environ 23(4):350–352

Nazina TN, Lebedeva EV, Poltaraus AB, Tourova TP, Grigoryan AA, Sokolova D, Lysenko AM, Osipov GA (2004) *Geobacillus gargensis* sp. nov., a novel thermophile from a hot spring, and the reclassification of *Bacillus vulcani* as *Geobacillus vulcani* comb. nov. Int J Syst Evol Microbiol 54(Pt 6):2019–2024

Nazina TN, Sokolova D, Grigoryan AA, Shestakova NM, Mikhailova EM, Poltaraus AB, Tourova TP, Lysenko AM, Osipov GA, Belyaev SS (2005) *Geobacillus jurassicus* sp. nov., a new thermophilic bacterium isolated from a high-temperature petroleum reservoir, and the validation of the *Geobacillus* species. Syst Appl Microbiol 28(1):43–53

Nazina TN, Tourova TP, Poltaraus AB, Novikova EV, Grigoryan AA, Ivanova AE, Lysenko AM, Petrunyaka VV, Osipov GA, Belyaev SS, Ivanov MV (2001) Taxonomic study of aerobic thermophilic bacilli: descriptions of *Geobacillus subterraneus* gen. nov., sp. nov. and *Geobacillus uzenensis* sp. nov. from petroleum reservoirs and transfer of *Bacillus stearothermophilus, Bacillus thermocatenulatus, Bacillus thermoleovorans, Bacillus kaustophilus, Bacillus thermodenitrificans* to *Geobacillus* as the new combinations *G. stearothermophilus,* G. th. Int J Syst Evol Microbiol 51(Pt 2):433–446

Niehaus F, Bertoldo C, Kahler M, Antranikian G (1999) Extremophiles as a source of novel enzymes for industrial application. Appl Microbiol Biotechnol 51(6):711–729

Nicolaus B, Manca MC, Lama L, Esposito E, Gambacorta A (1995) Effects of growth temperature on the polar lipid pattern and fatty acid composition of seven thermophilic isolates from the Antarctic continent. Syst Appl Microbiol 18(1):32–36

Nichols CM, Lardiere SG, Bowman JP, Nichols PD, Gibson JAE, Guezennec J (2005) Chemical characterization of exopolysaccharides from Antarctic marine bacteria. Microb Ecol 49(4):578–589

Nwodo UU, Green E, Okoh AI (2012) Bacterial exopolysaccharides: functionality and prospects. Int J Mol Sci 13(11):14002–14015

Obeidat M, Khyami-Horani H, Al-Zoubi A, Otri I (2012) Isolation, characterization, and hydrolytic activities of *Geobacillus* species from Jordanian hot springs. Afr J Biotechnol 11(25):6763–6768

Ogg CD, Spanevello MD, Patel BKC (2013) Exploring the ecology of thermophiles from Australia's Great Artesian basin during the genomic era. In: Satyanarayana T, Littlechild J, Kawarabayasi Y (eds) Thermophilic microbes in environmental and industrial biotechnology: biotechnology of thermophiles. Springer, Dordrecht, pp 61–97

Ollis DL, Cheah E, Cygler M, Dijkstra B, Frolow F, Franken SM, Harel M, Remington SJ, Silman I, Schrag J et al (1992) The alpha/beta hydrolase fold. Protein Eng 5(3):197–211

Olusesan AT, Azura LK, Abubakar F, Hamid NSA, Radu S, Saari N (2009) Phenotypic and molecular identification of a novel thermophilic *Anoxybacillus* species: a lipase-producing bacterium isolated from a Malaysian hotspring. World J Microbiol Biotechnol 25(11):1981–1988

Ortiz EM, Berretta MF, Navas LE, Benintende GB, Amadio AF, Zandomeni RO (2015) Draft genome sequence of *Geobacillus* sp. Isolate T6, a thermophilic bacterium collected from a thermal spring in Argentina. Genome Announc 3(4):e00743-15

Ozdemir S, Okumus V, Ulutas MS, Dundar A, Akarsubası AT, Dumonted S (2015) Isolation of a novel thermophilic *Anoxybacillus flavithermus* SO- 13, production, characterization and industrial applications of its thermostable α-amylase. J Bioprocess Biotech 5(7):1–9. https://doi.org/10.4172/2155-9821.1000237

Özdemir S, Kılınç E, Poli A, Nicolaus B (2013) Biosorption of heavy metals (Cd^{2+}, Cu^{2+}, Co^{2+}, and Mn^{2+}) by thermophilic bacteria, *Geobacillus thermantarcticus* and *Anoxybacillus amylolyticus*: equilibrium and kinetic studies. Bioremed J 17(2):86–96

Padilha I, Carvalho L, Dias P, Grisi T, Silva F, Santos S, Araujo D (2015) Production and characterization of thermophilic carboxymethyl cellulase synthesized by Bacillus sp. growing on

sugarcane bagasse in submerged fermentation. Braz J Chem Eng 32(1):35–42. https://doi.org/10.1590/0104-6632.20150321s00003298

Panosyan HH (2017) Thermophilic bacilli isolated from Armenian geothermal springs and their potential for production of hydrolytic enzymes. Int J Biotech Bioeng 3(8):239–244

Panosyan H (2010) Phylogenetic diversity based on 16S rRNA gene sequence analysis of aerobic thermophilic endospore-forming bacteria isolated from geothermal springs in Armenia. Biol J Armenia 4(65):73–80

Panosyan H, Birkeland NK (2014) Microbial diversity in an Armenian geothermal spring assessed by molecular and culture-based methods. J Basic Microbiol 54(11):1240–1250

Panosyan H, Anzelmo G, Nicolaus B (2014) Production and characterization of exopolysaccharadies synthesized by geobacilli isolated from an Armenian geothermal spring. FEBS J 281(1):667

Pikuta E, Cleland D, Tang J (2003) Aerobic growth of *Anoxybacillus pushchinoensis* K1(T): emended descriptions of *A. pushchinoensis* and the genus *Anoxybacillus*. Int J Syst Evol Microbiol 53(Pt 5):1561–1562

Pikuta E, Lysenko A, Chuvilskaya N, Mendrock U, Hippe H, Suzina N, Nikitin D, Osipov G, Laurinavichius K (2000) *Anoxybacillus pushchinensis* gen. nov., sp. nov., a novel anaerobic, alkaliphilic, moderately thermophilic bacterium from manure, and description of *Anoxybacillus flavitherms* comb. nov. Int J Syst Evol Microbiol 50(Pt 6):2109–2117

Pinzon-Martinez DL, Rodriguez-Gomez C, Minana-Galbis D, Carrillo-Chavez JA, Valerio-Alfaro G, Oliart-Ros R (2010) Thermophilic bacteria from Mexican thermal environments: isolation and potential applications. Environ Technol 31(8-9):957–966

Poli A, Guven K, Romano I, Pirinccioglu H, Guven RG, Euzeby JP, Matpan F, Acer O, Orlando P, Nicolaus B (2012) *Geobacillus subterraneus* subsp. *aromaticivorans* subsp. nov., a novel thermophilic and alkaliphilic bacterium isolated from a hot spring in Sirnak, Turkey. J Gen Appl Microbiol 58(6):437–446

Poli A, Romano I, Cordella P, Orlando P, Nicolaus B, Ceschi Berrini C (2009) *Anoxybacillus thermarum* sp. nov., a novel thermophilic bacterium isolated from thermal mud in Euganean hot springs, Abano Terme, Italy. Extremophiles 13(6):867–874

Poli A, Romano I, Caliendo G, Nicolaus G, Orlando P, Falco A, Lama L, Gambacorta A, Nicolaus B (2006) *Geobacillus toebii* subsp. *decanicus* subsp. nov., a hydrocarbon-degrading, heavy metal resistant bacterium from hot compost. J Gen Appl Microbiol 52:223–234

Priya I, Dhar MK, Bajaj BK, Koul S, Vakhlu J (2016) Cellulolytic activity of thermophilic bacilli isolated from Tattapani hot spring sediment in North West Himalayas. Indian J Microbiol 56(2):228–231. https://doi.org/10.1007/s12088-016-0578-4

Radchenkova N, Vassilev S, Panchev I, Anzelmo G, Tomova I, Nicolaus B, Kuncheva M, Petrov K, Kambourova M (2013) Production and properties of two novel exopolysaccharides synthesized by a thermophilic bacterium *Aeribacillus pallidus* 418. Appl Biochem Biotechnol 171(1):31–43

Rao MB, Tanksale AM, Ghatge MS, Deshpande VV (1998) Molecular and biotechnological aspects of microbial proteases. Microbiol Mol Biol Rev 62(3):597–635

Reddy NS, Nimmagadda A, Sambasiva Rao KRS (2003) An overview of the microbial α-amylase family. Afr J Biotechnol 2:645–648

Rekadwad BN (2015) Characterization of amylase from industrially important thermophilic microorganism: *Geobacillus thermoleovorans* strain Rekadwadsis. Int J Life Sci Biotechnol Pharma Res 4(1):26–30

Rozanov AS, Logacheva MD, Peltek SE (2014) Draft genome sequences of *Geobacillus stearothermophilus* strains 22 and 53, isolated from the garga hot spring in the Barguzin River Valley of the Russian Federation. Genome Announc 2(6):e01205-14

Satapathy SS, Dutta M, Ray SK (2010) Higher tRNA diversity in thermophilic bacteria: a possible adaptation to growth at high temperature. Microbiol Res 165(8):609–616

Satyanarayana T, Sharma A, Mehta D, Puri AK, Kumar V, Nisha M, Joshi S (2012) Biotechnological applications of biocatalysts from the firmicutes *Bacillus* and *Geobacillus* species. In:

Satyanarayana T, Johri BN (eds) Microorganisms in sustainable agriculture and biotechnology. Springer, Dordrecht, pp 343–379
Sawle L, Ghosh K (2011) How do thermophilic proteins and proteomes withstand high temperature? Biophys J 101(1):217–227
Seifzadeh S, Hassan Sajedi R, Sariri R (2008) Isolation and characterization of thermophilic alkaline proteases resistant to sodium dodecyl sulfate and ethylene diamine tetraacetic acid from *Bacillus* sp. GUS1. Iran J Biotechnol 6(4):214–221
Scandurra R, Consalvi V, Chiaraluce R, Politi L, Engel PC (1998) Protein thermostability in extremophiles. Biochimie 80(11):933–941
Schäffer C, Franck WL, Scheberl A, Kosma P, McDermott TR, Messner P (2004) Classification of isolates from locations in Austria and Yellowstone National Park as *Geobacillus tepidamans* sp. nov. Int J Syst Evol Microbiol 54(Pt 6):2361–2238
Schneider D, Liu Y, Gerstein M, Engelman DM (2002) Thermostability of membrane protein helix-helix interaction elucidated by statistical analysis. FEBS Lett 532:231–236
Scholz T, Demharter W, Hensel R, Kandler O (1987) *Bacillus pallidus* sp. nov., a new thermophilic species from sewage. Syst Appl Microbiol 9:91–96
Shahinyan G (2015) Temperature and pH effect on lipase production and enzymatic activity of thermophilic bacilli isolated from Armenian geothermal springs. Sci Med J 10(1):9–15
Shahinyan G, Margaryan A, Panosyan H, Trchounian A (2017) Identification and sequence analyses of novel lipase encoding novel thermophillic bacilli isolated from Armenian geothermal springs. BMC Microbiol 17(103). https://doi.org/10.1186/s12866-017-1016-4
Sharma A, Pandey A, Shouche YS, Kumar B, Kulkarni G (2009) Characterization and identification of *Geobacillus* spp. isolated from Soldhar hot spring site of Garhwal Himalaya, India. J Basic Microbiol 49(2):187–194
Sharma R, Thakur V, Sharma M and Birkeland N-K (2013) Biocatalysis through thermostable lipases: adding flavor to chemistry, In Satyanarayana, T., Littlechild, J. & Kawarabayasi, Y. (eds.) Thermophilic microbes in environmental and industrial biotechnology: DordrechtSpringer : 905-927
Sharma P, Gupta S, Sourirajan A, Dev K (2015) Characterization of extracellular thermophillic cellulase from thermophilic *Geobacillus* sp. isolated from Tattapani Hot spring of Himachal Pradesh, India. Int JAdv Biotechnol Res (IJBR) 6(3):433–442
Sharma A, Adhikari S, Satyanarayana T (2007) Alkali-thermostable and cellulase-free xylanase production by an extreme thermophile *Geobacillus thermoleovorans*. World J Microbiol Biotechnol 23(4):483–490
Shih TW, Pan TM (2011) Stress responses of thermophilic *Geobacillus* sp. NTU 03 caused by heat and heat-induced stress. Microbiol Res 166(5):346–359
Sookkheo B, Sinchaikul S, Thannan H, Thongprasong O, Phutrakul S, Chen ST (2002) Proteomic analysis of a thermostable superoxide dismutase from *Bacillus stearothermophilus* TLS33. Proteomics 2(9):1311–1315
Stenesh J, McGowan GR (1977) DNA polymerase from mesophilic and thermophilic bacteria. I. Purification and properties of DNA polymerase from *Bacillus licheniformis* and *Bacillus stearothermophilus*. Biochim Biophys Acta 272:156–166
Sung MH, Kim H, Bae JW, Rhee SK, Jeon CO, Kim K, Kim JJ, Hong SP, Lee SG, Yoon JH, Park YH, Baek DH (2002) *Geobacillus toebii* sp. nov., a novel thermophilic bacterium isolated from hay compost. Int J Syst Evol Microbiol 52(Pt 6):2251–2255
Sunna A, Prowe SG, Stoffregen T, Antranikian G (1997) Characterization of the xylanases from the new isolated thermophilic xylan-degrading *Bacillus thermoleovorans* strain K-3d and *Bacillus flavothermus* strain LB3A. FEMS Microbiol Lett 148(2):209–216
Sutherland IW (1999) Polysaccharases for microbial exopolysaccharides. Carbohydr Polym 38(4):319–328
Synowiecki J (2010) Some applications of thermophiles and their enzymes for protein processing. Afr J Biotechnol 9:7020–7025

Takami H, Takaki Y, Chee GJ, Nishi S, Shimamura S, Suzuki H, Matsui S, Uchiyama I (2004) Thermoadaptation trait revealed by the genome sequence of thermophilic *Geobacillus kaustophilus*. Nucleic Acids Res 21:6292–6303

Tan H Alam M (2010) sRNAs are involved in high temperature adaptation of Geobacillus thermoleovorans. EMBO Symposium Non-coding genome, At Heidelberg, Germany

Tolner B, Poolman B, Konings WN (1997) Adaptation of microorganisms and their transport systems to high temperatures. Comp Biochem Physiol 118A:423–428

Tourney J, Ngwenya BT (2014) The role of bacterial extracellular polymeric substances in geomicrobiology. Chem Geol 386(Supplement C):115–132

Trent JD (1996) A review of acquired thermotolerance, heat-shock proteins, and molecular chaperones in archaea. FEMS Microbiol Rev 18(2-3):249–258

Trivedi S, Gehlot HS, Rao SR (2006) Protein thermostability in Archaea and Eubacteria. Genet Mol Res 5(4):816–827

Turner P, Mamo G, Karlsson EN (2007) Potential and utilization of thermophiles and thermostable enzymes in biorefining. Microbial Cell Fact 6(1):9. https://doi.org/10.1186/1475-2859-6-9

Uma Maheswar Rao JL, Satyanarayana T (2007) Improving production of hyperthermostable and high maltose-forming alpha-amylase by an extreme thermophile *Geobacillus thermoleovorans* using response surface methodology and its applications. Bioresour Technol 98(2):345–352

van de Vossenberg JL, Ubbink-Kok T, Elferink MG, Driessen AJ, Konings WN (1995) Ion permeability of the cytoplasmic membrane limits the maximum growth temperature of bacteria and archaea. Mol Microbiol 18(5):925–932

van Kranendonk MJ, Djokic T, Campbell KA, Deamer D, Damer B, Walter MR, Steller L, Ota T, Nakamura E, Tadbiri S (2017) An origin of life at terrestrial hot springs: support from early earth and implications for the search for life on mars. Astrobiology Science Conference 2017 (LPI Contrib. No. 1965)

Vardanyan G, Margaryan A, Panosyan H (2015) Isolation and characterization of lipase-producing bacilli from Tatev geothermal spring. Collection of Scientific Articles of YSU SSS 1.1(4): 33–36

Wang GX, Wang Y, Wu ZF, Jiang HF, Dong RQ, Li FY, Liu XL (2011a) Immunomodulatory effects of secondary metabolites from thermophilic *Anoxybacillus kamchatkensis* XA-1 on carp, Cyprinus carpio. Fish Shellfish Immunol 30(6):1331–1338

Wang Q, Cen Z, Zhao J (2015) The survival mechanisms of thermophiles at high temperatures: an angle of omics. Physiology (Bethesda) 30(2):97–106

Wang X, Gao R, Liao C, Meng Y, Tang X, Li J, Xu B, Huang Z (2011b) Isolation of *Geobacillus* sp. A27 and characterization of its α-amylase. Sci Technol Food Ind 32:151–155 (in Chinese with English abstract)

Wang W, Ma T, Zhang B, Yao N, Li M, Li G, Ma Z, Cheng J (2014) A novel mechanism of protein thermostability: a unique N-terminal domain confers heat resistance to Fe/Mn-SODs. Sci Rep 4(7284):1–9. https://doi.org/10.1038/srep07284

Weiner R, Langille S, Quintero E (1995) Structure, function and immunochemistry of bacterial exopolysaccharides. J Ind Microbiol 15:339–346

Yang Z, Zhang Y, Shen T, Xie Y, Mao Y, Ji C (2013) Cloning, expression and biochemical characterization of a novel, moderately thermostable GDSL family esterase from *Geobacillus thermodenitrificans* T2. J Biosci Bioeng 115(2):133–137

Yohandini H, Julinar, Muharni (2015) Isolation and phylogenetic analysis of thermophile community within Tanjung Sakti Hot Spring, South Sumatera, Indonesia. HAYATI J Biosci 22(3):143–148

Yumoto I, Hirota K, Kawahara T, Nodasaka Y, Okuyama H, Matsuyama H, Yokota Y, Nakajima K, Hoshino T (2004) *Anoxybacillus voinovskiensis* sp. nov., a moderately thermophilic bacterium from a hot spring in Kamchatka. Int J Syst Evol Microbiol 54(Pt 4):1239–1242

Zahoor S, Javed M, Babarl M (2016) Characterization of a novel hydrolytic enzyme producing thermophilic bacterium isolated from the Hot Spring of Azad Kashmir-Pakistan. Braz Arch Biol Technol 56:e16150622. https://doi.org/10.1590/1678-4324-2016150662

Zeigler R (2001) *Bacillus* genetic stock center catalog of strains (7th edn), Volume 3: The genus *Geobacillus*: 11

Zhang XQ, Zhang ZL, Wu N, Zhu XF, Wu M (2013) *Anoxybacillus vitaminiphilus* sp. nov., a strictly aerobic and moderately thermophilic bacterium isolated from a hot spring. Int J Syst Evol Microbiol 63(Pt 11):4064–4071

Zhang CM, Huang XW, Pan WZ, Zhang J, Wei KB, Klenk HP, Tang SK, Li WJ, Zhang KQ (2011) *Anoxybacillus tengchongensis* sp. nov. and *Anoxybacillus eryuanensis* sp. nov., facultatively anaerobic, alkalitolerant bacteria from hot springs. Int J Syst Evol Microbiol 61(Pt 1):118–122

Zhao S, Cao F, Zhang H, Zhang L, Zhang F, Liang X (2014) Structural characterization and biosorption of exopolysaccharides from *Anoxybacillus* sp. R4-33 isolated from radioactive radon hot spring. Appl Biochem Biotechnol 172(5):2732–2746

Zhu W, Cha D, Cheng G, Peng Q, Shen P (2007) Purification and characterization of a thermostable protease from a newly isolated *Geobacillus* sp. YMTC 1049. Enzyme Microb Technol 40:1592–1597