African Journal of Biotechnology Vol. 11(25), pp. 6763-6768, 27 March, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.3099
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Isolation, characterization, and hydrolytic activities of *Geobacillus* species from Jordanian hot springs

Maher Obeidat[1]*, Hala Khyami-Horani[2], Adeeb Al-Zoubi[3] and Ismael Otri[1]

[1]Department of Biotechnology, Faculty of Agricultural Technology, Balqa' Applied University, 19117, Al-Salt, Jordan.
[2]Department of Biological Sciences, Faculty of Science, University of Jordan, Amman 11942, Jordan.
[3]Regenerative Medicine at the University of Illinois, USA.

Accepted 28 February, 2012

The present study was conducted to isolate, identify, characterize and to determine the enzymatic activities of the thermophilic *Geobacillus* species from five Jordanian hot springs. Based on phenotypic characters, eight thermophilic isolates were identified and belonged to the genus *Geobacillus*. The *Geobacillus* isolates were abundant in all investigated hot springs. The optimal temperature for growth of the isolates was 60 to 65°C and the optimal pH was 6 to 8. Colonies were light yellow circular to rhizoid. The bacterial cells were Gram positive rods and endospore forming. All isolates produced amylase, caseinase, alkaline and acid phosphatases, esterase (C4), esterase lipase (C8), α-Galactosidase, β-Glucuronidase, β-Glucosidase, and *N*-Acetyl-β-glucosaminidase. Seven isolates produced leucine and valine arylamidases and five isolates produced naphthol-AS-B1-phsphohydrolase. Lipase (C14) activity from two isolates and α-chymotrypsin activity from three isolates were also detected. The phenotypic characterization of those isolates was confirmed by genotypic method using 16S rDNA sequence analysis. Maximal homology of all eight isolates to genus *Geobacillus* was observed. Five of these isolates showed greater than 98% homology with *Geobacillus stearothermophilus* and one isolate showed 100% homology with *Geobacillus thermoglucosidasius*. Therefore, 16S rRNA gene sequence analysis can be considered as a valuable genotypic tool for the identification and characterization of thermophilic bacteria at genus level. Moreover, enzymatic products of those isolates could receive considerable attention due to their potential applications in biotechnology.

Keywords: Thermophiles, *Geobacillus*, hydrolytic enzymes, hot spring, 16S rRNA.

INTRODUCTION

Microorganisms occupy all possible environments including habitats offering ideal conditions for growth and extreme environments. Extremophiles, thermophiles in particular, are getting recognition mainly due to their attractive attributes in biotechnology. The discovery of different life forms at elevated temperatures and the isolation of *Thermus aquaticus* bacterium producing *Taq* DNA polymerase, which has a commercial success because of its uses in polymerase chain reaction (PCR) technology, led scientists to isolate and identify microorganisms from the worldwide geothermal sources. Thermophilic microorganisms are adapted to grow at high temperatures and they are separated into three categories; moderate thermophiles, extreme thermophiles and hyperthermophiles (Baker et al., 2001; Bertoldo and Antranikian, 2002). Thus, they can be isolated from high temperature terrestrial and marine habitats including volcanically and geothermally heated hydrothermal vent systems (Bertoldo and Antranikian, 2002).

Interest in thermophilic bacteria from the genus *Bacillus* has clearly emerged during the past two decades because of their significant potential for biotechnological applications including their importance as sources of thermostable enzymes (such as proteases, amylases,

*Corresponding author. E-mail: obeidatgh@yahoo.com. Tel: +962775609847. Fax: +96253530469.

lipases, xylanases, cellulases and DNA restriction endonucleases), and other products of industrial interest (Bergquist and Morgan, 1992; Maugeri et al., 2001; Rainey et al., 1994; Sharp et al., 1992). Thermophilic bacilli grow best at temperatures between 45 and 70°C, and were isolated from different environments including hot springs, petroleum reservoirs, deep-sea hydrothermal vents and deep ocean-basin cores (Bae et al., 2005; Rahman et al., 2004).

Recently, Nazina et al. (2001) grouped Gram positive, rod-shaped, endospore-forming thermophilic bacilli into the genus *Geobacillus*. The *Geobacillus* species are widely distributed and readily isolated from different habitats (Meintanis et al., 2006; Nazina et al., 2001), with a continually increasing industrial interest for their thermostable gene products (Lama et al., 2004; Schallmey et al., 2004). Therefore, studying phylogenetic relations and diversity in this novel bacterial genus is not only a taxonomical concern, but also a necessity in order to exploit its biotechnological potential completely.

Jordan has a unique location which is rich and diverse extreme environments including the hypersaline Dead Sea, the lowest place on earth, in addition to hot springs. Therefore, the aim of the current study was to isolate, identify and characterize thermophilic *Geobacillus* bacteria from five hot springs in Jordan using phenotypic (morphological, biochemical and physiological characters) and genotypic methods (16S rRNA gene sequencing). Moreover, the hydrolytic activities of the obtained isolates were also identified.

MATERIALS AND METHODS

Isolation of bacteria

Water samples were collected from five main hot springs located in different areas in Jordan using 500 ml sterile thermal glass containers. The samples were collected under 40 cm below the surface away from the margin. Water samples were filtered through 0.45 µm membrane filter. The filtrate was re-suspended in 10 ml of sterile sample water. From each sample, 100 µl aliquot was plated by spreading on nutrient agar plates (five replicates) and incubated for 24 h at 50°C. Different colonies were selected and purified by subculturing, and then, were stored in nutrient broth containing 20% glycerol at -80°C until was used.

Phenotypic and physiological characterization of isolates

Colony and cell morphology were performed according to the standard protocols. The presence of catalase, oxidase, amylase and caseinase were investigated for each isolate (Harley and Prescott, 2002). In addition, several enzymatic activities of the isolates were determined by API-ZYM system (BioMerieux, USA). Optimal growth temperature was determined by incubating the isolates in nutrient broth medium at 30 to 80°C with 5°C interval. Optimal pH was tested in the range 4.0 to 12.0 in nutrient broth medium with an interval of 0.5 units. The effect of NaCl on the thermophilic bacteria growth was studied by incubating the bacterial isolates in nutrient broth medium containing 0.0 to 10% with 0.5% interval.

Genomic DNA extraction

Thermophilic bacterial cultures which were thought to belong to *Geobacillus* and the reference strain *Geobacillus stearothermophilus* derived from ATCC 7953 were inoculated into 20 ml of Luria Bertani (LB) broth and incubated at 150 rpm overnight at 60°C. Cultures were centrifuged at 14000 rpm for 5 min, cell pellets were washed two times with distilled water, then used for DNA isolation using Wizard® Genomic DNA purification kit (Promega, USA, part no. A1120) according to the manufacturer's instructions. Genomic DNA from overnight culture of *Escherichia coli* ATCC 25922 was also extracted by the kit.

PCR amplification of the 16S rRNA gene

The PCR amplification of the 16S rRNA genes from purified genomic DNA was performed according to Belduz et al. (2003) by using the forward primer UNI16S-L (5′-ATTCTAGAGTTTGATCATGGCTCA-3′) corresponding to positions 11 to 26 of *E. coli* 16S rRNA and the reverse primer UNI16S-R (5′-ATGGTACCGTGTGACGGGCGGTGTGTA-3′) corresponding to the complement of positions 1411 to 1393 of *E. coli* 16S rRNA. PCR reaction conditions were carried out according to Beffa et al. (1996).

Sequencing analysis

The sequences of 16S rRNA gene from PCR products of the thermophilic isolates, the reference strain *G. stearothermophilus* ATCC 7953 and *E. coli* ATCC 25922 were determined with an Applied Biosystems model 373A DNA sequencer by using the ABI PRISM cycle sequencing kit (Macrogen, Korea). The sequences were compared with those contained in the GenBank (Benson et al., 1999) by using a basic local alignment tool (BLAST) search (Altschul et al., 1990). The most closely related 16S rRNA gene sequences to our isolates were retrieved from the database. Retrieved sequences were then aligned and the phylogenetic tree was constructed by the use of DNAMAN 5.2.9 sequence analysis software. *E. coli* ATCC 25922 was used as the outgroup in the tree.

RESULTS

In the present study, the occurrence of thermophilic bacteria belonging to the genus *Geobacillus* was investigated in five main hot springs in Jordan (Table 1). The water temperatures ranged from 42.1 to 62.3°C, with pH ranging from 6.0 to 7.0. The highest viable total bacterial count (>400 cfu/ml) was found in Jordan-Himma and Ma`in-Romman Bath, whereas the lowest count (11.4 CFU/ml) was observed in Hammat Afra. Table 1 shows that *Geobacillus* was abundant (264 CFU/ml) in waters of Jordan-Himma constituting 53.2% of the total viable bacterial count. Whereas, Shuna-North had the lowest content (0.8 CFU/ml) of *Geobacillus* with a 3.2% of the total viable bacterial count.

Out of 36 bacterial isolates from hot springs, 20 were thermophilic (Table 2). Based on morphological, physiological, and biochemical properties (Table 3), eight isolates (JTA1, JTH1, JTS1, JTZ1, JTZ5, JTM1, JTM5, and JTM8) met the criteria of the thermophilic genus *Geobacillus*. Colonies were light yellow circular to rhizoid on nutrient agar; cells were Gram positive, rod-shaped arranged in single or short chains, motile and endospore-

Table 1. Abundance and diversity of thermophilic *Geobacillus* species in Jordanian thermal vents.

Thermal vent	Water temperature (°C)	Water pH	Total bacterial CFU[a] / ml	*Geobacillus* CFU/ml (%)
Hammat Afra	48.2	6.5	11.4	3.2 (28.1)
Jordan Himma	42.1	7.0	496	264 (53.2)
Shuna-North	54.5	6.6	24.8	0.8 (3.2)
Zara-Bani Hamida	54.1	6.3	342	60 (17.5)
Ma`in-Roman Bath	62.3	6.0	429	119 (27.7)

[a]CFU, Colony forming unit. The CFU/ml values for total bacteria and *Geobacillus* were calculated after incubation of nutrient agar plates (five replicates) at 50°C for 24 h.

Table 2. Recovery of thermophilic *Geobacillus* from different Jordanian thermal vents.

Thermal vent	No. of bacterial isolates	No. of thermophilic isolates (%)	No. of *Geobacillus* isolates (%)	Isolates code
Hammat Afra	4	1 (25.0)	1 (100)	JTA1
Jordan Himma	6	2 (33.3)	1 (50.0)	JTH1
Shuna-North	5	3 (60.0)	1 (33.3)	JTS1
Zara-Bani Hamida	10	7 (70.0)	2 (28.6)	JTZ1, JTZ5
Ma`in-Roman Bath	11	7 (63.6)	3 (42.9)	JTM1, JTM5, JTM8
Total	36	20 (55.6)	8 (40)	

Table 3. Growth characteristics and enzymatic activity of thermophilic *Geobacillus* isolates.

Geobacillus isolate	JTA1	JTH1	JTS1	JTZ1	JTZ5	JTM1	JTM5	JTM8
Temperature (optimum)	40-85 (65)	40-80 (60)	40-80 (60)	40-85 (65)	40-80 (60)	40-80 (60)	40-85 (65)	40-85 (65)
pH (optimum)	4-11 (6-8)	6-9 (6-8)	6-9 (6-8)	4-11 (6-8)	6-9 (7-8)	6-9 (6-8)	4-11 (6-8)	4-11 (6-8)
NaCl %	0-4%	0-4%	0-4%	0-4%	0-4%	0-4%	0-4%	0-4%
Catalase	+	+	+	+	+	+	+	+
Oxidaes	+	+	+	+	+	+	+	+
Amylase	+	+	+	+	+	+	+	+
Caseinase	+	+	+	+	+	+	+	+
API ZYM								
Alkaline phosphatase	+	+	+	+	+	+	+	+
Esterase (C4)	+	+	+	+	+	+	+	+
Esterase Lipase (C8)	+	+	+	+	+	+	+	+
Lipase (C14)	+	-	-	-	-	-	-	+
Leucine arylamidase	+	+	+	-	+	+	+	+
Valine arylamidase	+	+	+	-	+	+	+	+
Cystine arylamidase	-	-	-	-	-	-	-	-
Trypsin	-	-	-	-	-	-	-	-
α-Chymotrypsin	+	+	+	-	-	-	-	-
Acid phosphatase	+	+	+	+	+	+	+	+
Naphthol-AS-B1-phosphohydrolase	-	-	+	+	+	-	+	+
α-Galactosidase	+	+	+	+	+	+	+	+
β-Galactosidase	-	-	-	-	-	-	-	-
β-Glucuronidase	+	+	+	+	+	+	+	+
α-Glucosidase	-	-	-	-	-	-	-	-
β-Glucosidase	+	+	+	+	+	+	+	+
N-Acetyl-β-glucosaminidase	+	+	+	+	+	+	+	+
α-Mannosidase	-	-	-	-	-	-	-	-
β-Fucosidase	-	-	-	-	-	-	-	-

Table 4. The comparison of the 16S rDNA gene sequences of the obtained *Geobacillus* isolates with the 16S rDNA gene sequences in the GenBank.

Geobacillus Isolate[a]	Sequence		
	No. of nucleotides[b]	% identity[c]	Closest phylogenetic relative (GenBank accession No.)
JTA1	1388	96	*Geobacillus* sp. RSNPB7 (HM588147)
JTH1	1377	96	*Geobacillus* sp. RSNPB7 (HM588147)
JTS1	1353	100	*G. thermoglucosidasius* (ADNQ01000070)
JTZ1	1387	96	*Geobacillus* sp. RSNPB7 (HM588147)
JTZ5	1033	95	*G. kaustophilus* (NC006510)
JTM1	1374	97	*Geobacillus* sp. RSNPB7 (HM588147)
JTM5	1374	96	*Geobacillus* sp. RSNPB7 (HM588147)
JTM8	1487	95	*G. stearothermophilus* WCH1 (HQ143640)
G.s. ATCC	7953	97	*Geobacillus* sp. RSNPB7 (HM588147)

[a]G.s. ATCC is the reference strain *G. stearothermophilus* ATCC 7953. [b]The number of 16S rDNA nucleotides used for the alignment. [c]The percentage identity with the 16S rDNA sequence of the closest phylogenetic relative of thermophiles.

forming. Optimal temperature was 60 to 65°C and optimal pH was 6 to 8 (Table 3). All isolates were able to grow within the range of 0-4% salt concentration. All isolates produced catalase, oxidase, amylase, caseinase, alkaline and acid phosphatases, esterase (C4), esterase lipase (C8), α-Galactosidase, β-Glucuronidase, β-Glucosidase, and *N*-Acetyl-β-glucosaminidase (Table 3). Most of the isolates produced leucine arylamidase, valine arylamidase, naphthol-AS-B1-phsphohydrolase. Only one isolate (JTZ1) did not show leucine and valine arylamidases activity and three isolates (JTA1, JTH1, and JTM1) did not show naphthol-AS-B1-phosphohydrolase activity. Lipase (C14) was produced only from two isolates (JTA1 and JTM8) and α-chymotrypsin activity was observed in three isolates (JTA1, JTH1, and JTS1).

16S rRNA gene sequence of the isolates and the reference strain *G. stearothermophilus* ATCC 7953 was analyzed. The 16S rDNA of the isolates was amplified with UNI16S-L and UNI16S-R primers. The amplified genomic DNA of the isolates and the reference strain produced PCR band with about 1400 bp in size. Based on BLAST alignment of GenBank sequences to 16S rDNA sequences, all eight isolates belonged to genus *Geobacillus* with 95 to 100% identity (Table 4). The phylogenetic analysis of the 16S rDNA sequences reflected the affiliation of the isolates with the genus *Geobacillus* and showed that five isolates (JTA1, JTH1, JTZ1, JTM1, and JTM5) appeared closely related to the reference strain (*G. stearothermophilus* ATCC 7953) with 98.9 to 100% homology and one isolate (JTM8) had 95.8% homology with *G. stearothermophilus* WCH1 (HQ143640) (Figure 1 and Table 5). Whereas, JTS1 and JTZ5 isolates showed 100 and 95.7% homology with *Geobacillus thermoglucosidasius* (ADNQ01000070) and *Geobacillus kaustophilus* (NC006510), respectively.

DISCUSSION

A total of 20 thermophilic bacterial isolates were isolated from five hot springs in Jordan. Based on the phenotypic and physiological properties such as occurrence of endospores, production of enzymes and growth at high temperature, a total of eight isolates appeared to be closer to the genus *Geobacillus*. In terms of phenotypic and physiological characters, the identification of those isolates as *Geobacillus* species is in agreement with findings of previous studies (Ezeji et al., 2005; Fortina et al., 2001; Nazina et al., 2001, 2004; Romano et al., 2005).

The 16S rDNAs of the isolates and one reference strain (*G. stearothermophilus* ATCC 7953) were sequenced. Therefore, the preliminary identification of the obtained bacterial isolates by conventional methods was confirmed by comparing the 16S rDNA sequences of the isolates with those in the GenBank. As a result, the sequences of the obtained isolates in this study showed 95.7 to 100% sequence homology to the genus *Geobacillus*. These results are consistent with Nazina et al. (2001), who reported that members of the genus *Geobacillus* have more than 96% sequence homology. Considering phylogenetic analysis using 16S rDNA, it was revealed that five *Geobacillus* isolates could be allocated into the species *stearothermophilus* with homology values ranging between 98.9 to 100%. Moreover, the isolate JTM8 had the highest homology (95.7%) with the species *stearothermophilus*. On the other hand, JTS1 and JTZ5 isolates could be allocated into the species *thermoglucosidasius* (100% homology) and *kaustophilus* (95.7% homology). However, this needs to be further confirmed by fatty acid analysis, DNA hybridization and other techniques. While these results are important for

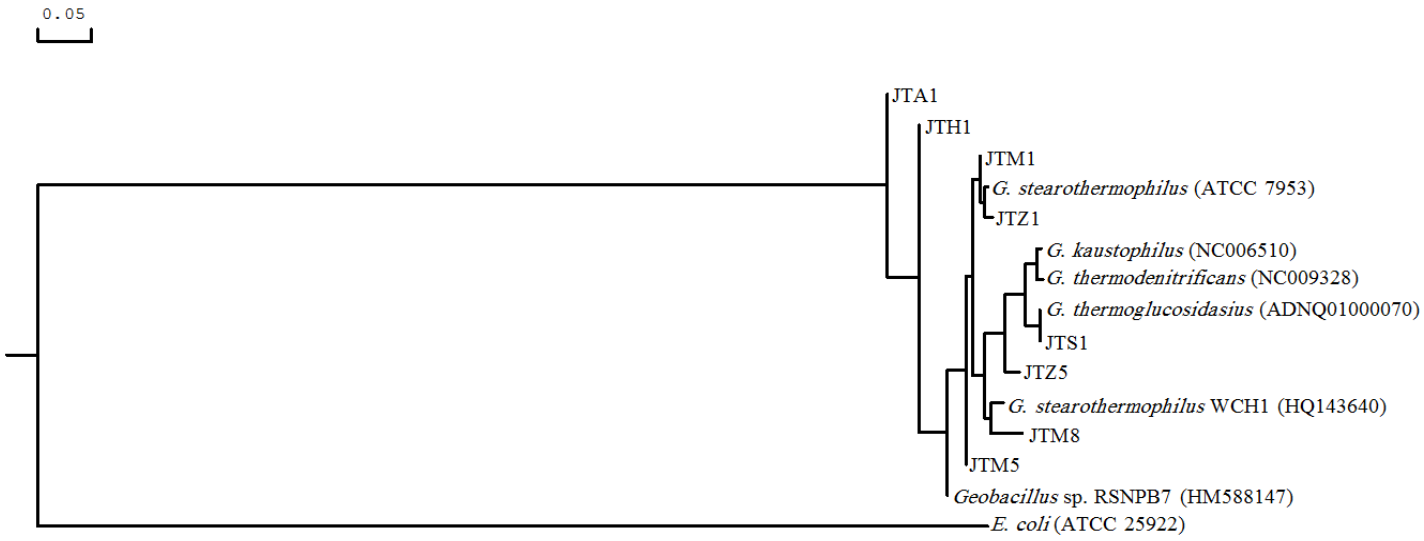

Figure 1. Phylogenetic tree showing the relationships between the 16S rDNA sequences of the eight *Geobacillus* isolates, the reference strain *G. stearothermophilus* ATCC 7953 and other related *Geobacillus* sequences previously published in the database. The accession numbers are given in parentheses. The phylogenetic tree was built by the neighbor-joining method, using maximum likelihood parameter distance from the partial 16S rDNA sequences. *E. coli* (ATCC 25922) was used as the outgroup.

Table 5. Homology matrix shared between Jordanian *Geobacillus* isolates, the reference strain *G. stearothermophilus* ATCC 7953, and other related *Geobacillus* sequences previously published in the database.

Isolate[a]	1	2	3	4	5	6	7	8	9	10	11	12	13
2	92.1%												
3	99.6%	92.5%											
4	99.3%	92.5%	99.3%										
5	94.5%	92.1%	94.5%	96.1%									
6	98.5%	92.1%	99.6%	98.2%	93.7%								
7	94.3%	95.0%	95.2%	95.7%	93.4%	94.3%							
8	100%	92.1%	99.6%	99.3%	94.5%	98.5%	94.3%						
9	100%	92.1%	99.6%	99.3%	94.2%	98.9%	94.3%	100%					
10	92.7%	100%	92.9%	92.7%	91.8%	91.5%	95.0%	92.7%	92.7%				
11	92.5%	96.9%	93.2%	92.3%	91.3%	91.3%	95.7%	92.5%	92.5%	97.1%			
12	92.2%	97.2%	92.9%	92.2%	91.5%	91.3%	95.5%	92.2%	92.2%	97.2%	98.8%		
13	98.3%	91.6%2	97.9%	97.6%	94.3%	96.8%	93.9%	98.3%	98.3%	91.9%	91.6%	91.4%	
14	95.2%	92.1%	95.7%	96.8%	95.8%	95.2%	94.6%	95.2%	95.2%	92.8%	93.1%	92.8%	95.2%

[a]1, JTH1; 2, JTS1; 3, JTM1; 4, JTM5; 5, JTM8; 6, JTZ1; 7, JTZ5; 8, JTA1; 9, *G. stearothermophilus* ATCC 7953; 10, *G. thermoglucosidasius* (ADNQ01000070); 11, *G. kaustophilus* (NC006510); 12, *G. thermodenitrificans* (NC009328); 13, *Geobacillus* sp. RSNPB7 (HM588147); and 14, *G. stearothermophilus* WCH1 (HQ143640).

further taxonomic work, positive results on several enzymes such as amylase, caseinase, lipase, phosphatases and arylamidases of most isolates as well as chymotrypsin activity in some isolates are indication of potential applications of these bacterial products in biotechnology. Therefore, those isolates could receive considerable attention mainly due to the production of industrially important enzymes.

It was clearly observed that all Jordanian hot springs investigated in this study were found rich in *Geobacillus* species. This richness could be correlated to the environmental conditions and the nutritional status available for growth of *Geobacillus* in waters of such hot springs.

ACKNOWLEDGEMENTS

The authors are grateful to the Scientific Research Support Fund / Ministry of Higher Education of Jordan for

the financial support.

REFERENCES

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990). Basic local alignment search tool. J. Mol. Biol. 215: 403-410.

Bae SS, Lee JH, Kim SJ (2005). *Bacillus alveayuensis* sp. nov., a thermophilic bacterium isolated from deep-sea sediments of the Ayu Trough. Int. J. Syst. Evol. Microbiol. 55: 1211-1215.

Baker G, Gaffar S, Cowan DA, Suharto AR (2001). Bacterial community analysis of Indonesian hotsprings. FEMS Microbiol. Lett. 200: 103-109. Beffa T, Blanc M, Lyon PF, Vogt G, Marchiani M, Fischer JL, Aragno M (1996). Isolation of *Thermus* strains from hot composts (60 to 80 °C). Appl. Environ. Microbiol. 62: 1723-1727.

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: 1315-1320.

Benson DA, Boguski MS, Lipman DJ, Oullette BFF, Rapp BA, Wheelet DL (1999). GenBank. Nucleic Acids Res. 27: 12-17.

Bergquist PC, Morgan HW (1992). The molecular genetics and biotechnological application of enzyme from extremely thermophilic eubacteria. In: Herbert RA, Sharp RJ (eds) Molecular biology and biotechnology of extremophiles. Chapman and Hall, New York, pp. 44-45.

Bertoldo C, Antranikian G (2002). Starch-hydrolyzing enzymes from thermophilic archea and bacteria. Curr. Opin. Chem. Biol. 6: 151-160.

Ezeji TC, Wolf A, Bahl H (2005). Isolation, characterization, and identification of *Geobacillus thermodenitrificans* HRO10, an α-amylase and α-glucosidase producing thermophile. Can. J. Microbiol. 51(8): 685-693.

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. 5: 2063-2071.

Harley JP, Prescott LM (2002). Laboratory exercises in microbiology, 5th edn. The McGraw-Hill Companies, New York.

Lama L, Calandrelli V, Gambacorta A, Nicolaus B (2004). Purification and characterization of thermostable xylanase and beta-xylosidase by the thermophilic bacterium *Bacillus thermantarcticus*. Res. Microbiol. 155: 283-289.

Maugeri TL, Gugliandolo C, Caccamo D, Stackebrandt E (2001). A polyphasic taxonomic study of thermophilic bacilli from shallow, marine vents. Syst. Appl. Microbiol. 24: 572-587.

Meintanis C, Chalkou KI, Kormas KA, Karagouni AD (2006). Biodegradation of crude oil by thermophilic bacteria isolated from a volcano island. Biodegradation, 17: 105-111.

Nazina TN, Lebedeva EV, Poltaraus AB, Tourova TP, Grigoryan AA, Sokolova DS, 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: 2019-2024.

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 thermoglucosidasius* and *Bacillus thermodenitrificans* to *Geobacillus* as the new combinations *G. stearothermophilus, G. thermocatenulatus, G. thermoleovorans, G. kaustophilus, G. thermoglucosidasius* and *G. thermodenitrificans*. Int. J. Syst. Evol. Microbiol. 51: 433-446.

Rahman TJ, Marchant R, Banat IM (2004). Distribution and molecular investigation of highly thermophilic bacteria associated with cool soil environments. Biochem. Soc. Trans. 32: 209-213.

Rainey FA, Fritze D, Stackebrandt E (1994). The phylogenetic diversity of thermophilic members of the genus *Bacillus* as revealed by 16S rDNA analysis. FEMS Microbiol. Lett. 115: 205-212.

Romano I, Poli A, Lama L, Gambacorta A, Nicolaus B (2005). *Geobacillus thermoleovorans* subsp. *stromboliensis* subsp. nov., isolated from the geothermal volcanic environment. J. General. Appl. Microbiol. 51: 183-189.

Schallmey M, Singh A, Ward OP (2004). Developments in the use of *Bacillus* species for industrial production. Can. J. Microbiol. 50: 1-17.

Sharp RJ, Riley PW, White D (1992). Heterotrophic thermophilic bacilli. In: Kristjansson JK (ed) Thermophilic Bacteria. CRC Press, Boca Raton, pp. 19-50.