INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

TAXONOMIC DESCRIPTION
Najar *et al.*, *Int J Syst Evol Microbiol* 2018;68:3430–3434
DOI 10.1099/ijsem.0.003002

MICROBIOLOGY SOCIETY

Geobacillus yumthangensis sp. nov., a thermophilic bacterium isolated from a north-east Indian hot spring

Ishfaq Nabi Najar,[1] Mingma Thundu Sherpa,[1] Sayak Das,[1] Kamalesh Verma,[2] Vikash Kumar Dubey[2,3] and Nagendra Thakur[1,*]

Abstract

A thermophilic, spore-forming, rod-shaped bacterium isolated from the Yumthang hot spring in North Sikkim, India was subjected to taxonomic studies. The thermophilic bacterial isolate was designated as strain AYN2^T. Cells were Gram-stain-positive, aerobic, motile, rod-shaped, catalase-positive and methyl red-negative. Strain AYN2^T was able to grow in the pH range from 6 to 10 (optimum, pH 7.5–8.0), at 40–70 °C (60 °C) and in NaCl concentrations of 0–4 % (1 %). The major cellular fatty acids were iso-$C_{15:0}$ (12.8 %), iso-$C_{16:0}$ (13.9 %) and iso-$C_{17:0}$ (13.8 %). No matches were found in the RTSBA6 Sherlock libraries. The G+C content of the genomic DNA was 42.11 mol%. Based on phylogenetic analysis of the 16S rRNA gene sequences, strain AYN^T showed highest sequence similarity to the type strain of *Geobacillus toebii* (96 %). However, the phenotypic properties of strain AYN2^T were clearly distinct from those of *G. toebii* and related species. On the basis of polyphasic analysis, strain AYN2^T represents a novel species in the genus *Geobacillus*, for which the name *Geobacillus yumthangensis* sp. nov. is proposed. The type strain is AYN2^T(MTCC=12749=KCTC=33950= JCM 32596).

In 1991, based on 16S rRNA sequences, Ash *et al.* analysed many species of *Bacillus* and further investigations found that they fell into five distinct groups [1]. Subsequently, using phylogenetic, physiological and morphological characteristics led to the description of *Geobacillus* [2]. With high levels of 16S rRNA sequence similarity of 98.5–99.2 %, the *Geobacillus* species include a cogent group of thermophilic bacilli *(Bacillus sterothermophilus*, *Bacillus thermoleovorans*, *Bacillus thermocatenulatus*, *Bacillus kaustophillus*, *Bacillus thermodenitrificans* and *Bacillus thermoglucosidasius*). *Geobacillus* species are endospore-forming, Gram-stain-positive, aerobic or facultative anaerobic rods [3]. They normally grow within the range of temperatures from 35 to 80 °C, depending on the strain [4]. The *Geobacillus* species can be found in various harsh environments that include high-temperature oilfields [5], marine vents [6], corroded pipeline in an extremely deep well [4], African [7] and Russian [8] hot springs, and the Mariana Trench [9]. In addition, they can also be found in hay compost [10] and garden soil [11]. Their thermostable characteristics make members of *Geobacillus* attractive to the biotechnology industry as sources of thermostable enzymes [12], as platforms for biofuel production [13] and as attractive constituents of bioremediation strategies [14]. Here we describe a novel species of the genus *Geobacillus,* which can grow in wide range of pH.

Water samples were taken from the source of the Yumthang hot spring, North Sikkim, India (27° 47′ 34.50″ N 88° 42′ 30.96″ E). The *in situ* temperature and pH values were measured using a Multiparameter Water Quality Checker U-50 Series (Horiba). The temperature of the source was between 42–45 °C and the pH values ranged from pH 7.5 to 8. Strain AYN2^T was isolated on solid thermus agar containing 8 g l^{-1} peptone, 4 g l^{-1} yeast extract and 2 g l^{-1} NaCl with an incubation of 24 h at 60 °C. Strain AYN2^T was also able to grow on actinomycetes agar with the composition of 2 g l^{-1} sodium caseinate, 0.1 g l^{-1} L-asparagine, 4 g l^{-1} sodium propionate, 0.5 g l^{-1} dipotassium phosphate, 0.1 g l^{-1} magnesium sulphate, 0.001 g l^{-1} ferrous sulphate and 25 g l^{-1} agar. The colonies were round and creamy-white coloured. The Gram-stain reaction and spore staining were performed using a Gram-staining kit (Difco) and a spore-staining kit, respectively, following the recommended protocols. Strain AYN2^T was found to be a spore-forming Gram-stain-

Author affiliations: [1]Department of Microbiology, School of Life Sciences, Sikkim University, 6th Mile, Samdur, Tadong, Gangtok – 737102, Sikkim, India; [2]Centre for the Environment, Indian Institute of Technology Guwahati, Guwahati-781039, Assam, India; [3]Department of Biosciences and Bioengineering, Indian Institute ofTechnology Guwahati, Guwahati-781039, Assam, India.
***Correspondence:** Nagendra Thakur, nthakur@cus.ac.in
Keywords: *Geobacillus yumthangensis* sp. nov.; thermophiles; hot spring; polyphasic taxonomy; Sikkim.
Abbreviations: FESEM, field emission scanning electron microscope; SEM, scanning electron microscope; KCTC, Korean Collection for Type Cultures; MTCC, Microbial Type Culture Collection; JCM, Japan Collection of Microorganisms; FAME, Fatty Acid Methyl Ester; PCR, Polymerase Chain Reaction.
The GenBank accession numbers for the 16S rRNA gene and the whole genome are MG603320 and NWUZ00000000, respectively.
Two supplementary figures and two supplementary tables are available with the online version of this article.

positive bacteria. Scanning electron microscope (SEM) analysis, i.e. field emission scanning electron microscopy (FESEM), was performed as per Hagen *et al.* [15, 16] using a Sigma FESEM (Zeiss). The SEM analysis showed that cells were 2.5–5 µm long and 0.4–0.6 µm wide (Fig. S1, available in the online version of this article).

The effect of temperature, pH and NaCl on growth of bacteria was determined as per Aaniz *et al.* [17]. Strain AYN2^T could grow between 40 and 70 °C with the optimal temperature of 60 °C and in the pH ranges from 6 to 10 with the optimum pH of 7.5–8.0 at the optimum growth temperature. The NaCl concentration for the growth of strain AYN2^T was found to be in the range between 0 and 4%, with the optimum NaCl concentration of 1 % at the optimum pH and temperature, as shown in Fig. S2(a–c). By comparing these results with the closely related species of *Geobacillus*, especially *Geobacillus toebii* (the closest one), it was found that there is considerable distinction between them. For example, if we compare the pH ranges, strain AYN2^{T}grows over a wide range of pH, i.e. from pH 6–10 as compared to other *Geobacillus* species which thus adds to its novelty (Table 1). Also, many biochemical characteristics such as: production of acids from various sugars such as ribose, inositol, glycerol and cellobiose; hydrolysis of casein, gelatin and starch; utilization of acetate, formate and lactate; and fermentation of glucose are quite different from those of *G. toebii* and other related species as shown in Table 1. All of the phenotypic and biochemical tests were performed manually or by using the Biolog system.

The fatty acid analysis of 16 h grown culture of strain AYN2^{T}was performed at 50 °C. The fatty acid extraction and analysis were performed by following the instructions of the Microbial Identification System (MIDI) as reported previously [18]. The RTSBA6 method was used and the

Table 1. Phenotypic characteristics that differentiate *Geobacillus yumthangensis* sp. nov. strain AYN2^T from its phylogenetic neighbours

Strains: 1, AYN2^T (manual); 2,AYN2^T (Biolog); 3, *Geobacillus toebii* [10]; 4, *Geobacillus thermoglucosidasius* [32]; 5, *Geobacillus uzenensis* [2]; 6, *Geobacillus subterraneus* [2]; 7, *Geobacillus stearothermophilus* [33]; 8, *Geobacillus thermocatenulatus* [34]; 9, *Geobacillus thermoleovorans* [35]; 10, *Geobacillus kaustophilus* [36]; 11, *Geobacillus thermodenitrifcans* [37]. +, Positive; −, negative; D, 11±89 % of strains positive; ND, not determined.

	1	2	3	4	5	6	7	8	9	10	11
Cell width (µm)	0.4–0.6	ND	0.5–0.9	<3	0.9–1.3	0.8–1.5	0.6–1	0.5–1.2	0.9	1.5	0.5–1.0
Cell length (µm)	2.5–5	ND	2–3.5	<0.9	4.7–8	4.7–8	2–3.5	3.0–7.0	6.0–8	3.5	1.5–2.5
Motility	+	ND	+	ND	+	+	+	+	+	−	ND
Production of acid from:											
Adonitol	ND	ND	−	+	−	−	ND	−	ND	ND	ND
L-Arabinose	−	ND	−	−	+	−	D	−	−	D	+
Cellobiose	+	+	−	+	+	+	−	+	+	+	+
Galactose	−	+	−	D	+	+	−	−	+	+	+
Ribose	+	ND	−	−	+	+	ND	ND	ND	+	+
Glycerol	ND	+	−	−	+	+	+	+	+	D	+
Inositol	−	+	+	+	−	−	−	−	−	−	ND
Lactose	−	+	−	−	−	−	−	−	−	−	+
Rhamnose	−	+	−	−	−	−	−	+	−	−	−
Sorbitol	−	+	−	−	−	−	−	+	ND	−	ND
D-Xylose	−	ND	−	+	−	−	D	+	−	D	+
Hydrolysis of:											
Gelatin	−	+	−	+	+	−	D	−	−	ND	ND
Casein	−	−	+	+	−	−	D	+	ND	+	−
Starch	+	+	−	+	+	+	+	+	−	D	+
Aesculin	−	ND	−	−	+	+	ND	+	ND	ND	ND
Utilization of:											
Formate	ND	+	−	D	−	+	−	ND	ND	ND	ND
Acetate	ND	+	−	−	+	+	−	ND	ND	ND	ND
Lactate	ND	+	−	−	+	+	−	ND	ND	ND	ND
Citrate	−	−	−	+	−	−	D	D	+	ND	ND
Fermentation of glucose	+	+	−	−	−	−	D	−	+	−	ND
Methyl red test	−	ND	−	−	−	+	D	D	ND	ND	ND
Denitrification	ND	ND	+	ND	−	+	−	−	+	ND	+
NaCl concentration for growth (%, w/v)	0–5	0–5	0–5	0–5	0–4	0–5	0–5	0–1.5	0–4	ND	0–3
pH range for growth	6.0–10	>6.0	6.0–9.0	6.0–8.0	6.2–7.8	6.0–7.8	6.0–8.0	6.5–8.5	6.2–7.8	6.2–7.5	6.0–8.0
Temperature range for growth (°C)	40–70	ND	45–70	37–68	45–65	45–70	37–65	42–69	35–78	40–75	45–70

Table 2. Fatty acid composition of strain AYN2^T and type strains of *Geobacillus* with validly published names

Strains: AYN2^T; *Geobacillus toebii* [10]; *Geobacillus thermodenitrificans* [2]; *Geobacillus thermoleovorans* [2]; *Geobacillus thermocatenulatus* [2]; *Geobacillus stearothermophilus* [38]; *Geobacillus thermoglucosidasius* [38]. The values in bold signifies the major fatty acids present in individual bacterial strains.

	iso-C14 : 0	C14 : 0	iso-$C_{15:0}$	a-$C_{15:0}$	$C_{15:0}$	iso-$C_{16:0}$	$C_{16:0}$	Iso-$C_{17:0}$	a-$C_{17:0}$	C-$_{17:0}$	Iso-$C_{18:0}$	$C_{18:1}$	$C_{18:0}$	References
G. yumthangensis AYN2^T	–	3.3	**12.8**	–	–	**13.9**	7.0	**13.7**	3.2	4.7	2.3	3.2	2.6	This paper
G. toebii	–	–	**34.0**	–	–	**17.0**	–	**34.0**	–	–	–	–	–	[10]
G. thermodenitrificans	0.4	1.8	**33.6**	1.8	2.3	9.5	**11.0**	**26.6**	7.3	2.9	0.2	1.3	1.3	[2]
G. thermoleovorans	1.0	1.4	**22.6**	1.3	2.1	**21.0**	**11.2**	**18.5**	4.6	1.3	0.9	1.2	3.4	[2]
G. thermocatenulatus	1.3	0.6	**25.5**	0.6	1.3	**31.8**	8.3	**21.0**	3.1	2.3	1.3	0.7	2.2	[2]
G. stearothermophilus	0.1	1.5	**39.8**	6.4	0.5	6.2	9.2	**17.1**	**13.3**	–	–	–	–	[38]
G. thermoglucosidasius	–	0.6	**22.0**	1.6	–	**10.4**	**11.6**	**30.3**	**16.6**	0.8	–	–	–	[38]

results were analysed by using Sherlock version 6.2. The predominant fatty acids were iso-$C_{15:0}$ (12.8 %), iso-$C_{16:0}$ (13.9 %) and iso-$C_{17:0}$ (13.7 %). The results of a comparison between the various fatty acids of strain AYN2^T and its closest relatives are shown in Table 2. Although the major fatty acids are similar, the total amount or the percentage of total fatty acids vary considerably. Thus these fatty acid profiles supports the inclusion of strain AYN2^T in the genus *Geobacillus*. When analysing the results, no matches were found in the RTSBA6 Sherlock libraries, thus suggesting that strain AYN2^T is novel.

The 16S rRNA sequence was amplified by using universal primers (27F and 1492R) as described by Hugenholtz [19] and the product was purified by using the QIAquick PCR purification kit (Qiagen). The purified 16S rRNA gene was further sequenced by using the BigDye Terminator cycle sequencing kit (version 3.1, Applied Biosystems) in an automated DNA Sequencer (ABS/Genetic 3500 Analyzer). The sequences were assembled by CodonCode Aligner (version 7.1) and the assembled sequences were identified by performing BLAST searches. Strain AYN2^T shared only 96 % identity with the *G. toebii* strain R-35642, which confirms the novelty of strain AYN2^T. The 16S rRNA gene sequence of strain AYN2^T was aligned with representative 16S rRNA gene sequences of related taxa using CLUSTAL_W software [20]. A phylogenetic tree was reconstructed by using the neighbour-joining method [21] and the software package MEGA 7 to demonstrate the relationship of strain AYN2^T to other members of the family *Geobacillus* (Fig. 1). Also, three more conserved genes, i.e. *rpoB, DnaK* and *dnaJ* genes, were investigated using primers *rpoB*1698F (5′-AACATCGGTTTGATCAAC-3′; corresponding to *E. coli* position 1643) and *rpoB*2041R (5′-CGTTGCATGTTGGTACCCAT-3′; corresponding to *E. coli* position 2041) [22]; *dnaK*F (5′-CTCCGTGGACCTTCTCTTGG-3′) and *dnaK*R (5′-ATGATCTGCTTGTGGGCCTC-3′) [23]; and *dnaJ*F (5′-CAGATCGAGGTSACCTTCGAC-3′) and *dnaJ*R (5′-CGTCRYCATMGAGATCGGCAC-3′) [24]. After performing BLAST searches for the *rpoB* gene, the results showed 88 % identity with *G. thermoglucosidasius*. The heat shock chaperone genes, i.e. *dnaK* and *dnaJ*, showed 89 and 90 % identity with *G. thermoglucosidasius* and *Bacillus thermoglucosidasius* respectively. Thus, these lower values shown for the *rpoB*, *Dnak* and *DnaJ* genes support AYN2^T being a novel species.

The draft genome sequence of the species *Geobacillus yumthangensis* AYN2^T has been published recently [25]. The whole genome accession numbers are given in Table S1. RAST (version 2.0) [26] was employed, which annotated 962 proteins. The genome of AYN2^T was 3.4 Mbp and contained 3721 predicted genes [25]. The number of rRNA genes and tRNA genes were 5 and 71, respectively. The absence of any photosynthetic apparatus and gene clusters indicated that strain AYN2^T may be adapted to a heterotrophic metabolism. The G+C content of AYN2^{T}was also checked using Valvet (version 1.1.10) and SSPACE (version 3.0) in our previous study [25]. The G+C content was found to be 42.11 mol%, which is quite different from the other species of genus *Geobacillus* (Table S4). Whole genome sequencing results [25] showed that the novel organism does not possess any plasmid, as compared to the other closest *Geobacillus* species such as *G. thermoglucosidasius* [27], *G. sp,* WCH70 [28], *G. stearothermophilus* [29], etc. The original methods of determining average nucleotide identity and average nucleotide identity of orthologous genes were calculated using the Orthologous Average Nucleotide Identity Tool (version 0.93) [30]. The original average nucleotide identity and the average nucleotide identity of orthologous genes between strain AYN2^T and the closest species, *G. toebii*, were 97.6 and 97.8 %, respectively. Digital DNA–DNA hybridization values were determined using the Genome-to-Genome Distance Calculator (version 2.1) [31]. The digital DNA–DNA hybridization values were 69.10 %. Although the average nucleotide identity and digital DNA–DNA hybridization values were a little higher or comparable to the cut-off values of ~96 and <70 %, respectively. However, the cell morphology, biochemical characteristics, sugar fermentation, 16S rRNA, *rpoB*, *dnaJ* and

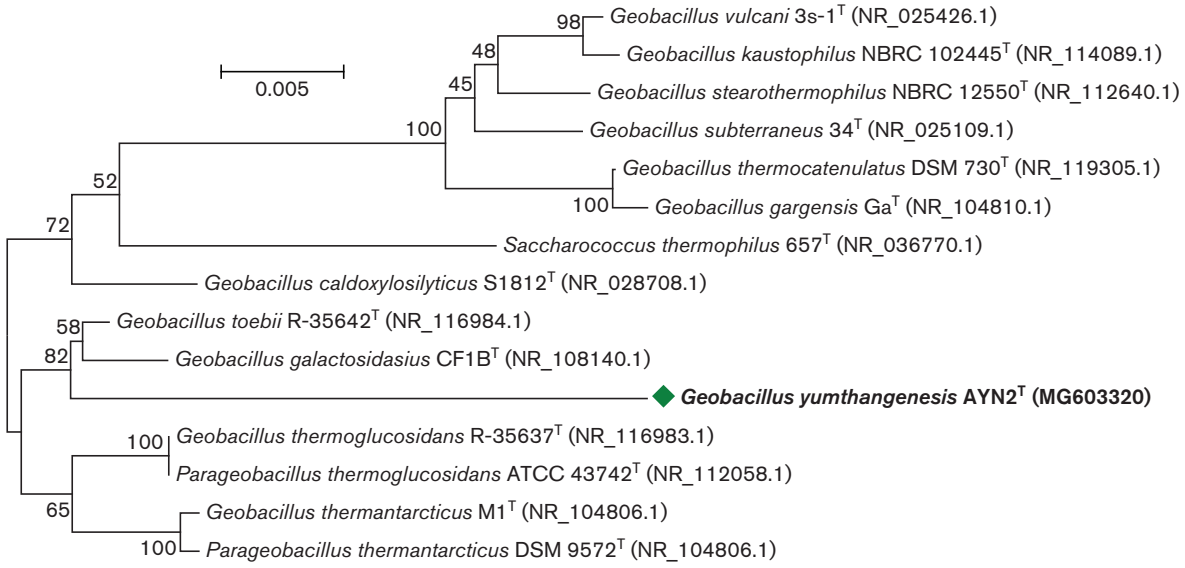

Fig. 1. Phylogenetic tree showing the position of strain AYN2^T among *Geobacillus* species and related taxa. The evolutionary history was inferred using the neighbor-joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches The evolutionary distances were computed using the maximum-composite-likelihood method and are in the units of the number of base substitutions per site. Evolutionary analyses were conducted in MEGA7.

dnaK gene similarity, G+C content, absence of plasmids, and fatty acid methyl ester analysis suggested that strain AYN2^T represents a novel species.

DESCRIPTION OF *GEOBACILLUS YUMTHANGENSIS* SP. NOV.

Geobacillus yumthangensis (yum.thang.en.sis.N.L.masc.adj. *yumthangensis* of Yumthang referring to Yumthang Hot Spring from where the type strain was isolated).

Cells are Gram-stain-positive, aerobic, rod-shaped, 2.5–5 µm long and 0.4–0.6 µm wide. They form endospores located at the sub-terminal to terminal positions. Growth is observed between 40 and 70 °C with the optimum temperature of 60 °C. The pH for growth ranges from pH 6 to 10 with the optimum pH of 7.5–8 at the optimum growth temperature. The NaCl concentration for the growth of AYN2^T is in the range between 0 and 5%, with the optimum NaCl concentration of 1 % at the optimum pH and temperature. No growth is observed at 80 °C and at pH below 6 and above 10. No growth is observed at concentrations of 5 % NaCl and above. Cells are catalase-positive and methyl red-negative. Able to utilize lactate, formate and acetate. Acids are produced from cellobiose, lactose, galactose, sorbitol and glucose, but not from arabinose, ribose or xylose. The major cellular fatty acids are iso-$C_{15:0}$, iso-$C_{16:0}$ and iso-$C_{17:0}$. The G+C content is 42.11 mol%. The isolate was isolated from Yumthang hot spring, North Sikkim, India. The type strain is strain AYN2^T (MTCC=12749=KCTC=33950=JCM=32596) (Table S3).

Funding information
The work was funded by the Department of Biotechnology, Government of India (BCIL/NER-BPMC/2016). The authors will also like to acknowledge I.I.T. Guwahati for providing the FESEM at the Central Instrumentation Laboratory.

Acknowledgements
The authors would like to thank the Department of Forest, Government of Sikkim for their kind permission and the Department of Microbiology, Sikkim University for laboratory space.

Conflicts of interest
The authors declare that there are no conflicts of interest.

References

1. **Ash C, Farrow JAE, Wallbanks S, Collins MD.** Phylogenetic heterogeneity of the genus *Bacillus* revealed by comparative analysis of small-subunit-ribosomal RNA sequences. *Lett Appl Microbiol* 1991;13:202–206.
2. **Nazina TN, Tourova TP, Poltaraus AB, Novikova E V, Grigoryan AA *et al.*** 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* 2001;51:433–446.
3. **Zeigler DR.** The *Geobacillus* paradox: why is a thermophilic bacterial genus so prevalent on a mesophilic planet? *Microbiology* 2014; 160:1–11.

4. Popova NA, Nikolaev I, Turova TP, Lysenko AM, Osipov GA *et al.* *Geobacillus uralicus*, a new species of thermophilic bacteria. *Mikrobiologiia* 2002;71:335–341.
5. Kuisiene N, Raugalas J, Chitavichius D. *Geobacillus lituanicus* sp. nov. *Int J Syst Evol Microbiol* 2004;54:1991–1995.
6. Maugeri TL, Gugliandolo C, Caccamo D, Stackebrandt E. Three novel halotolerant and thermophilic *Geobacillus* strains from shallow marine vents. *Syst Appl Microbiol* 2002;25:450–455.
7. Hawumba JF, Theron J, Brözel VS. Thermophilic protease-producing *Geobacillus* from Buranga hot springs in Western Uganda. *Curr Microbiol* 2002;45:144–150.
8. Nazina TN, Lebedeva EV, Poltaraus AB, Tourova TP, Grigoryan AA *et al.* *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* 2004;54:2019–2024.
9. Takami H, Nishi S, Lu J, Shimamura S, Takaki Y. Genomic characterization of thermophilic *Geobacillus* species isolated from the deepest sea mud of the Mariana Trench. *Extremophiles* 2004;8:351–356.
10. Sung MH, Kim H, Bae JW, Rhee SK, Jeon CO *et al.* *Geobacillus toebii* sp. nov., a novel thermophilic bacterium isolated from hay compost. *Int J Syst Evol Microbiol* 2002;52:2251–2255.
11. Wiegand S, Rabausch U, Chow J, Daniel R, Streit WR *et al.* Complete genome sequence of *Geobacillus* sp. Strain GHH01, a thermophilic lipase-secreting bacterium. *Genome Announc* 2013;1:1–2.
12. de Champdoré M, Staiano M, Rossi M, D'Auria S. Proteins from extremophiles as stable tools for advanced biotechnological applications of high social interest. *J R Soc Interface* 2007;4:183–191.
13. Cripps RE, Eley K, Leak DJ, Rudd B, Taylor M *et al.* Metabolic engineering of *Geobacillus thermoglucosidasius* for high yield ethanol production. *Metab Eng* 2009;11:398–408.
14. Markossian S, Becker P, Märkl H, Antranikian G. Isolation and characterization of lipid-degrading *Bacillus thermoleovorans* IHI-91 from an icelandic hot spring. *Extremophiles* 2000;4:365–371.
15. Hagen CA, Hawrylewicz EJ, Anderson BT, Tolkacz VK, Cephus ML. Use of the scanning electron microscope for viewing bacteria in soil. *Appl Microbiol* 1968;16:932–934.
16. Golding CG, Lamboo LL, Beniac DR, Booth TF. The scanning electron microscope in microbiology and diagnosis of infectious disease. *Sci Rep* 2016;6:1–8.
17. Aanniz T, Ouadghiri M, Melloul M, Swings J, Elfahime E *et al.* Thermophilic bacteria in Moroccan hot springs, salt marshes and desert soils. *Braz J Microbiol* 2015;46:443–453.
18. Buyer JS, Sasser M. High throughput phospholipid fatty acid analysis of soils. *Applied Soil Ecology* 2012;61:127–130.
19. Hugenholtz P, Pitulle C, Hershberger KL, Pace NR. Novel division level bacterial diversity in a Yellowstone Hot Spring novel division level bacterial diversity in a Yellowstone Hot Spring. *J Bacteriol* 1998;180:366–376.
20. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 1994;22:4673–4680.
21. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4:406–425.
22. Dahllöf I, Baillie H, Kjelleberg S. rpoB-based microbial community analysis avoids limitations inherent in 16S rRNA gene intraspecies heterogeneity. *Appl Environ Microbiol* 2000;66:3376–3380.
23. Cusick KD, Fitzgerald LA, Cockrell AL, Biffinger JC. Selection and evaluation of reference genes for reverse transcription-quantitative pcr expression studies in a thermophilic bacterium grown under different culture conditions. *PLoS One* 2015;10:e0131015–e0131023.
24. Diouf F, Diouf D, Klonowska A, Le Queré A, Bakhoum N *et al.* Genetic and genomic diversity studies of *Acacia* symbionts in Senegal reveal new species of Mesorhizobium with a putative geographical pattern. *PLoS One* 2015;10:e0117667.
25. Najar IN, Sherpa MT, Das S, Thakur N. Draft genome sequence of *Geobacillus yumthangensis* AYN2 sp. nov., a denitrifying and sulfur reducing thermophilic bacterium isolated from the hot springs of Sikkim. *Gene Rep* 2018;10:162–166.
26. Aziz RK, Bartels D, Best AA, Dejongh M, Disz T *et al.* The RAST Server: rapid annotations using subsystems technology. *BMC Genomics* 2008;9:75.
27. Brumm P, Land ML, Hauser LJ, Jeffries CD, Chang Y-J *et al.* Complete genome sequence of *Geobacillus* strain Y4.1MC1, a novel coutilizing *Geobacillus thermoglucosidasius* strain isolated from bath hot spring in Yellowstone National Park. *Bioenergy Res* 2015;8:1039–1045.
28. Brumm PJ, Land ML, Mead DA. Complete genome sequences of *Geobacillus* sp. WCH70, a thermophilic strain isolated from wood compost. *Stand Genomic Sci* 2016;11:1–8.
29. Stuknyte M, Guglielmetti S, Mora D, Kuisiene N, Parini C *et al.* Complete nucleotide sequence of pGS18, a 62.8-kb plasmid from *Geobacillus stearothermophilus* strain 18. *Extremophiles* 2008;12:415–429.
30. Lee I, Ouk Kim Y, Park SC, Chun J. OrthoANI: An improved algorithm and software for calculating average nucleotide identity. *Int J Syst Evol Microbiol* 2016;66:1100–1103.
31. Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 2013;14:60.
32. Suzuki Y, Kishigami T, Inoue K, Mizoguchi Y, Eto N *et al.* *Bacillus thermoglucosidasius* sp. nov., a new species of obligately thermophilic Bacilli. *Syst Appl Microbiol* 1983;4:487–495.
33. Burgess SA, Flint SH, Lindsay D, Cox MP, Biggs PJ. Insights into the *Geobacillus stearothermophilus* species based on phylogenomic principles. *BMC Microbiol* 2017;17:1–12.
34. Golovacheva RS, Loginova LG, Salikhov TA, Kolesnikov AA, Zaĭtseva GN. [New species of thermophilic bacilli–*Bacillus thermocatenulatus* nov. sp]. *Mikrobiologiia* 1975;44:265–268.
35. Zarilla KA, Perry JJ. *Bacillus thermoleovorans*, sp. nov., a species of obligately thermophilic hydrocarbon utilizing endospore-forming bacteria. *Syst Appl Microbiol* 1987;9:258–264.
36. White D, Sharp RJ, Priest FG. A polyphasic taxonomic study of thermophilic bacilli from a wide geographical area. *Antonie van Leeuwenhoek* 1994;64:357–386.
37. Manachini PL, Mora D, Nicastro G, Parini C, Stackebrandt E *et al.* *Bacillus thermodenitrificans* sp. nov., nom. rev. *Int J Syst Evol Microbiol* 2000;50:1331–1337.
38. Kämpfer P. Limits and possibilities of total fatty acid analysis for classification and identification of *Bacillus* species. *Syst Appl Microbiol* 1994;17:86–98.