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TAXONOMIC DESCRIPTION
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Bacillus pasinlerensis sp. nov., a thermophilic bacterium isolated from a hot spring in Turkey

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Abstract

A Gram-reaction-positive, endospore-forming bacterium, designated strain P1^T, was isolated from water samples collected from Pasinler Hot Spring and characterized using a polyphasic approach to clarify its taxonomic position. Strain P1^T was found to have chemotaxonomic and morphological characteristics consistent with its classification in the genus *Bacillus*. The strain shared the highest 16S rRNA gene sequence identity values with *Bacillus thermolactis* R-6488^T (97.6%) and *Bacillus kokeshiiformis* MO-04^T (97.2%) and formed a distinct clade with both type strains in the phylogenetic trees based on 16S rRNA gene sequences. Strain P1^T could grow optimally at 55 °C and in the presence of 2% NaCl. The organism was found to contain meso-diaminopimelic acid as the diagnostic diamino acid in the cell-wall peptidoglycan. The major polar lipids were diphosphatidylglycerol and phosphatidylglycerol. The predominant menaquinone was determined to be MK-7. The major cellular fatty acids were identified as iso-$C_{15:0}$, iso-$C_{17:0}$ and anteiso-$C_{17:0}$. Based upon the consensus of phenotypic and phylogenetic analyses, strain P1^T represents a novel species of the genus *Bacillus*, for which the name *Bacillus pasinlerensis* sp. nov. is proposed. The type strain is P1^T (=DSM 107529^T=CECT 9885^T=NCCB 100674^T).

The genus *Bacillus*, firstly described by Cohn (1872) [1], comprises rod-shaped, endospore-forming bacteria, which can be isolated from diverse habitats such as compost [2], desert [3], human gut [4], lake [5, 6], marine sediment [7], rhizosphere [8] and soil [9–11]. *Bacillus* species are mainly characterized by the presence of meso-diaminopimelic acid in the cell-wall peptidoglycan and MK-7 as major menaquinone [12]. Members of the genus show various physiological characteristics including strains with acidophilic [13], alkalitolerant [14], halophilic [15] and thermophilic growth [16–19]. Recently, this heterogenous genus has undergone a comprehensive phylogenomic reclassification, which has resulted in emergence of six novel genera from the genus *Bacillus* [20].

In recent years, considerable attention has been focused on isolation of novel bacteria from extreme habitats such as hot springs [21–23]. In the present study, we isolated a thermophilic bacterium from a hot spring in Turkey and further characterized it as a novel species of the genus *Bacillus*, for which *Bacillus pasinlerensis* is proposed.

ISOLATION AND ECOLOGY

The water samples were collected from a hot spring (39° 54′ 59″ N 41° 37′ 30″E) in Pasinler, Erzurum (Turkey) and used as the source of isolation (01 December 2017). The temperature, pH and depth of the hot spring are 39–45 °C, 6.5 and 205 m, respectively. The water samples were filtered within 2 h of sampling through Millipore membrane filters with a pore size of 0.45 µm. The filters were transferred on Tryptic Soy Agar (TSA) and incubated aerobically at 55 °C for 24 h. The sample suspensions were then diluted 100-fold, and 100 µl of samples was spread on TSA plates (peptone from casein, 15 gl^{-1}; peptone from soymeal, 5 gl^{-1}; NaCl, 5 gl^{-1}; agar, 15 gl^{-1}) and incubated at 55 °C for 48 h under aerobic conditions. Strain P1^T, a circular, cream-coloured, smooth colony, was

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Keywords: *Bacillus*; hot spring; thermophilic; new species; polyphasic taxonomy.
Abbreviations: HPLC, high-performance liquid chromatography; ML, maximum-likelihood; MP, maximum-parsimony; R2A, Reasoner's 2A; TLC, thin layer chromatography.
The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene and draft genome sequence of strain P1^T are MG768952 and JAACYS000000000, respectively.
Three supplementary figures are available with the online version of this article.

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selected and pure cultures were obtained after performing three successive transfers to fresh media. The strain was maintained on TSA plates at 4 °C and in 20% (v/v) glycerol stock solutions at −86 °C.

16S RNA PHYLOGENY

The genomic DNA of the strain was extracted using Wizard Genomic DNA Purification Kit (Promega) and PCR amplification of the 16S rRNA gene was achieved using the universal primers 27F (5′-AGAGTTTGATCCTGGCTCA-3′) and 1492R (5′- GGTTACCTTGTTACGACTT-3′) [24]. The PCR products were cloned into pGEM-T Easy Vector (Promega UK) and then sequenced at Macrogen (The Netherlands) and an almost complete 16S rRNA gene sequence (1424 bp) was deposited in the GenBank data library. The pairwise sequence analyses of the 16S rRNA gene were conducted on EzBioCloud server [25] available at https://www.ezbiocloud.net/ and also on GGDC web server available at https://ggdc.dsmz.de/. The corresponding sequences of the closely related type species were also retrieved from EzBioCloud server. A multiple sequence alignment was generated using MUSCLE algorithm [26] and a neighbour-joining tree [27] was constructed in MEGA X [28] using the model of Jukes and Cantor [29]. Moreover, phylogenetic analyses for maximum-likelihood (ML) and maximum-parsimony (MP) methods were conducted by the GGDC web server using the DSMZ phylogenomics pipeline [30] adapted to single genes after pairwise sequence similarities were calculated according to Meier-Kolthoff *et al.* [31] via the GGDC web server [32] available at http://ggdc.dsmz.de/. The ML tree was inferred from the alignment with RAxML [33] using rapid bootstrapping in conjunction with the autoMRE criterion [34] and the MP tree was inferred from the alignment with TNT [35] using 1000 bootstraps in conjunction with tree-bisection-and-reconnection branch swapping and ten random-sequence additional replicates. The sequences were checked for compositional bias using the χ^2 test as implemented in PAUP* [36].

The pairwise sequence analysis of the 16S rRNA gene of strain P1^T showed that the strain had high sequence identity with *Bacillus thermolactis* R-6488^T (97.6%) and *Bacillus kokeshiiformis* MO-04^T (97.2%). Strain P1^T formed a distinct clade with both type species in the phylogenetic trees based on neighbour-joining (Fig. 1) and ML algorithms (Fig. S1, available in the online version of this article). DNA–DNA hybridization assays were not carried out between strain P1^T and its closely related neighbours as the shared 16S rRNA gene sequence similarity was found to be well below thresholds (98.7–99%) considered to trigger such studies [32, 37, 38].

GENOME FEATURES

The whole genome of strain P1^T was sequenced using the Illumina HiSeq 2500 next-generation sequencing platform with a 250 bp paired-end protocol (MicrobesNG, Birmingham, UK) and the draft genome sequence was submitted to NCBI database under the accession number JAACYS000000000. The assembled genome sequence was checked for possible contaminations using the ContEST16 tool available at https://www.ezbiocloud.net/tools/contest16s [39]. The genome sequence was annotated on the Rapid Annotations Using Subsystems Technology (RAST) server [40]. Predictions of the secondary metabolite coding gene clusters were achieved on antiSMASH web server [41].

The draft genome is composed of 176 contigs with N50 value of 32.848 and coverage of 90×. The numbers of coding sequences and RNAs are 3330 and 84, respectively. The total genome size is 3189538 bp and the G + C content is 34.8%, which is close to the lower boundary of 32–66% reported for the genus *Bacillus* [12] while consistent with the values reported for the closest phylogenetic type species of the strain [2, 42]. According to antiSMASH analysis, strain P1^T has a lanthipeptide-coding gene cluster with 80% similarity to geobacillin I, an antimicrobial lanthipeptide from *Geobacillus thermodenitrificans* NG80-2 [43].

PHYSIOLOGY AND CHEMOTAXONOMY

Cultural and morphological characteristics of the strain were observed using established cultivation conditions at 55 °C. The type species *Bacillus thermolactis* R-6488^T and *Bacillus kokeshiiformis* MO-04^T were obtained from German Collection of Microorganisms and Cell Cultures (DSMZ, Germany) for comparative analyses. Gram-reaction was carried out following the method described by Gerhardt *et al.* [44] and endospore formation was determined by observation with transmission electron microscope (Fig. S3b) and malachite green staining of cells grown on R2A agar for 48 h (Fig. S3c) [45]. The motility was determined by observing dispersed growth of cells in test tubes containing semi-solid TSA agar (0.3%, w/v) and photomicrograph of cell with flagella was taken by TEM (Fig. S3a) [46].

In order to determine temperature range for growth, the strain was grown in Tryptic Soy Broth (TSB) at 25, 30, 35, 40, 45, 50, 55, 60, 65 and 70 °C for 48 h. After the optimum temperature (55 °C) was determined. Growth at various NaCl concentrations (0–10%, at intervals of 1.0%) was determined at the optimal temperature of the test strain in TSB medium lacking NaCl, and NaCl was added as a supplement at various concentrations. The pH range for growth was determined (4.0–11.0 at 0.5 unit intervals, by using the appropriate biological buffers: Na_2HPO_4/NaH_2PO_4 for below pH 8.0; $Na_2CO_3/NaHCO_3$ for pH 8.0–10.0; and $Na_2HPO_4/NaOH$ for pH 11.0) in TSB medium. All parameters (temperature, salt concentration and pH value) were performed in triplicate and measured at OD_{600} nm. Anaerobic growth feature was tested as described by Schäffer [47]. Growth abilities of the strain P1^T were tested on various media including TSA (Pancreatic Digest of Casein 15.0 gl^{-1}, Peptic Digest of Soybean Meal 5.0 gl^{-1}, Sodium Chloride 5.0 gl^{-1}, Agar 15.0 gl^{-1}) Nutrient agar (Peptone 5.0 gl^{-1},Yeast Extract 2.0 gl^{-1}, Sodium Chloride 5.0 gl^{-1}, Agar 15.0 gl^{-1}) Luria–Bertani agar (Tryptone 10.0 gl^{-1}, Yeast extract 5.0 gl^{-1}, NaCl 10.0 gL^{-1}, Agar 15.0 gl^{-1}) and Plate Count agar (Enzymatic Digest of Casein/tryptone

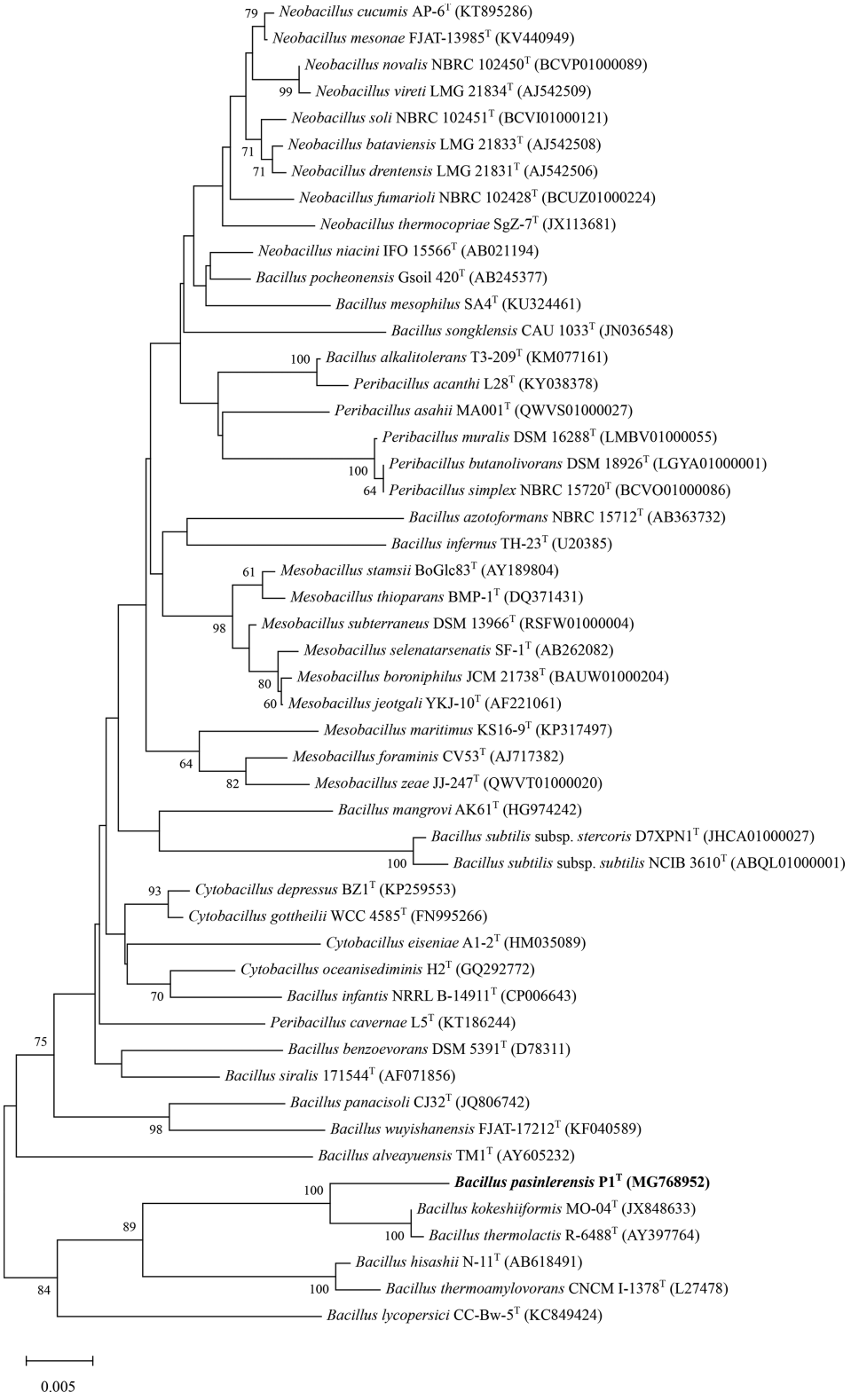


Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences of strain P1^T and related type species of the genus *Bacillus* as well as type species formerly classified in the genus *Bacillus*. Numbers at the nodes represent bootstrap values. The evolutionary distances were computed using the Jukes–Cantor method [29] and are in the units of the number of base substitutions per site. The analysis involved 50 nucleotide sequences. All positions containing gaps and missing data were eliminated (complete deletion option). There were a total of 1152 positions in the final dataset. Only bootstrap values more than≥60% are shown. Bar indicates 0.005 substitutions per site.

5.0 gl^{-1}, Yeast Extract 2.5 gl^{-1}, Glucose 1.0 gl^{-1}, Agar 15.0 gl^{-1}). Maximum growth for the strain was detected on TSA media. However, growth on Nutrient Agar, LB Agar and Plate Count Agar was weak. Strain P1^T could not grow under anaerobic conditions. Strain P1^T could grow at 40–60 °C (optimum at 55 °C) and pH 6–10 (optimum pH 7). The strain could tolerate NaCl up to 3.0% (w/v) and optimum growth was observed in the presence of 2% NaCl.

Oxidase reagent (Sigma 70439) was used for testing oxidase activity. Catalase activity was observed by the production of bubbles after the addition of a drop of 3% (v/v) H_2O_2. Hydrolysis of starch and casein were carried out on modified TSA supplemented with 1% soluble starch, 1% casein, respectively. Arginine dihydrolase, ornithine decarboxylase, Voges–Proskauer test, production of indole, hydrolysis of aesculin and p-nitrophenyl-b-D-galactopyranoside (pNPG) and the assimilation of carbon sources were tested with the API 20 E system (bioMerieux) as recommended by the manufacturer. Nitrate reductase, urease and gelatinase activities were also determined using the API 20 E system. Production of other enzymes was assessed using the API ZYM system (bioMerieux). Acid production from different carbohydrates was investigated using the API 50CH system (bioMerieux). To prevent the API strips from drying out, they were placed in a container with sterilized water and then incubated. Sterile water was added when needed [48]. All API tests were performed in triplicate. Strain P1^T was able to hydrolyse aesculin, casein and starch. The strain could produce acid from ribose, D-glucose, a-methyl-D-glucosamine, fructose, maltose, melezitose, sucrose and trehalose. Detailed physiological characteristics of the strain are given in Table 1 and species description.

Biomass for chemotaxonomic analyses was obtained by cultivation of the strain in shake flasks at 55 °C for 3 days on a rotary shaker (150 r.p.m.) using tryptic soy broth (TSB). Biomass was harvested by centrifugation, washed with sterile distilled water and then freeze-dried. Whole cell amino acids were extracted following a standard protocol described by Lechevalier and Lechevalier [49] and analysed by thin-layer chromatography (TLC) according to Staneck and Roberts [50]. Polar lipids were extracted and analysed by two-dimensional TLC following Minnikin *et al.* [51] using modifications of Kroppenstedt and Goodfellow [42]. Menaquinones were extracted from freeze-dried biomass and purified according to Collins [52]. The extracted menaquinones were analysed using high-performance liquid chromatography (HPLC; LC-10AS, Shimadzu 63 Co.) fitted with a reverse-phase C18 column (150×4.6 mm, 5 µm particle size, RP-18-Lichrosorb column, Penomenex UK) at 270 nm. Fatty acid methyl esters were extracted from fresh cultures grown on TSA at 55 °C for 24 h and analysed using Microbial Identification System (MIDI) according to the technical instructions provided by this system [53].

Chemotaxonomic characteristics of strain P1^T were consistent with those of members of the genus *Bacillus*. The peptidoglycan of strain P1^T contained meso-DAP as the cell-wall

Table 1. Differential phenotypic characteristics between strain P1^T and phylogenetically related species. All experiments were performed under identical conditions at the same time

Strains: 1, P1^T; 2, *Bacillus thermolactis* R-6488^T; 3, *Bacillus kokeshiiformis* MO-04^T. +: Positive; w: weakly positive; –, negative.

Characteristic	1	2	3
Acid production from:			
L-Arabinose	–	+	+
D-Xylose	–	+	+
L-Xylose	–	+	–
Galactose	–	–	+
Fructose	+	+	–
Sorbose	–	+	+
Aesculin	–	–	+
Cellulose	–	–	+
Maltose	+	–	+
Sucrose	+	–	–
Trehalose	+	–	+
Melezitose	+	–	–
Turanose	w	–	–
Tagatose	w	w	–
Hydrolysis of:			
Starch	+	–	–
Aesculin	+	w	+
Casein	+	+	–
Growth with NaCl (%) at:			
1	+	–	+
3	+	–	+
8	–	–	+
Growth at pH:			
4.5	–	–	+
9	+	–	+
Growth at temperature:			
30 °C	–	–	+
60 °C	+	–	+
Enzyme activity (API ZYM)			
Alkaline phosphatase	–	+	+
Esterase (C4)	–	+	+

Continued

Table 1. Continued

Characteristic	1	2	3
DNA G+C content (mol%)	34.8	35.0*	33.4†
Isolation source	Hot spring	Raw milk*	Marine animal resource compost†

*Data taken from Coorevits *et al*. [54].
†Data taken from Paudel *et al*. [2].

diaminoacid. The polar lipid profile was found to contain diphosphatidylglycerol, phosphatidylglycerol, a glycophospholipid, two glycolipids, three unidentified phospholipids and three unidentified lipids (Fig. S2). The predominant menaquinone was determined to be MK-7 (95%). The major cellular fatty acids were identified as iso-$C_{15:0}$ (36.5%), iso-$C_{17:0}$ (15.0%) and anteiso-$C_{17:0}$ (12.5%) (Table 2).

A comparative polyphasic analysis between strain P1^T and closely related type species *B. thermolactis* R-6488^T and *B. kokeshiiformis* MO-04^T revealed that the strain could be distinguished from the closely related type species on the basis of chemical, molecular and physiological characteristics, including hydrolysis of starch, acid production from melezitose and sucrose as well as the presence of glycolipids in polar lipid profile. Considering also the latest reclassification of the genus *Bacillus* based on whole-genome data [20], strain P1^T was clustered together with the members of the genus *Bacillus* in phylogenetic trees supported by high bootstrap values. However, further phylogenomics analyses encompassing all type strains within the genus are required to resolve the taxonomic heterogeneity of the genus *Bacillus*. In conclusion, based on morphological, chemotaxonomical and phylogenetic analyses, strain P1^T represents a novel member of the genus *Bacillus*, for which *Bacillus pasinlerensis* sp. nov. is proposed.

Table 2. Fatty acid profiles of strain P1^T and its phylogenetic neighbours. The strains were grown under identical conditions. All data are from this study

	Strain P1^T	*B. thermolactis* R-6488^T	*B. kokeshiiformis* MO-04^T
Saturated			
$C_{14:0}$	3.3	1.0	1.6
$C_{16:0}$	7.9	4.0	5.3
$C_{17:0}$	3.5	2.7	5.4
$C_{18:0}$	3.4	–	1.6
$C_{20:0}$	–	–	1.0
Unsaturated			
$C_{16:1}$ ω7c	–	1.4	1.3
$C_{18:0}$ 10- *methyl*	–	–	1.7
$C_{17:1}$ ω9c	–	–	1.4
Branched			
iso $C_{14:0}$	3.0	1.3	–
iso $C_{15:0}$	**36.5**	**36.2**	**31.8**
iso $C_{16:0}$	7.9	**18.8**	**13.4**
iso $C_{17:0}$	**14.9**	**24.4**	**24.4**
iso $C_{17:1}$ ω10c	–	1.2	1.4
anteiso $C_{15:0}$	7.0	2.1	2.0
anteiso $C_{17:0}$	**12.5**	5.0	5.6

PROTOLOGUE

Bacillus pasinlerensis (pa.sin.ler.en'sis. N.L. masc. adj. *pasinlerensis* of Pasinler, where the type strain was isolated). The Gram-stain-positive, oxidase and catalase positive, aerobic, motile, rod-shaped cells are 0.4–0.6×2.0–5.0 µm in size. Ellipsoidal endospores are formed terminally. Growth occurs at 40–60 °C (optimum, 55 °C), pH 6.0–10.0 (optimum, pH 7.0) and with 0–3% (w/v) NaCl (optimum, 2%). Aesculin, casein and starch are hydrolysed but urea is not hydrolysed. Glucose is utilized but, citrate, tryptophane, D-mannitol, Inositol, D-sorbitol, L-rhamnose, sucrose, melibiose, Amygdalin and L-arabinose are not utilized. Positive for gelatinase, naphthol-AS-BI-phosphohydrolase and β-glucosidase activities but negative for O-nitrophenyl-b-D-galactopyranoside, arginine dihydrolase, Lysine Decarboxylase, Ornithine Decaboxylase, alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14) leucine arylamidase, trypsin, chymotrypsin, acid phosphatase, valine arylamidase, cystine arylamidase α-galactosidase, β-galactosidase, α-glucosidase, *N*-acetyl-b-glucosaminidase, α-fucosidase, β-glucuronidase and a-mannosidase activities. Voges–Proskauer test is negative, H_2S and Indole production are absent. In the API 50CHB gallery, high quantities of acid are produced from ribose, D-glucose, D-fructose, maltose, melezitose, sucrose, a-methyl-D-glucosamine and trehalose. Acid is not produced from glycerol, erythritol, D-arabinose, L-arabinose, D-xylose, L-xylose, adonitol, b-methyl-D-xyloside, D-galactose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, amethyl-D-mannoside, D-mannose, amygdalin, lactose, inulin, raffinose, xylitol, gentiobiose, D-fucose, L-fucose, D-arabitol, melibiose, gluconate, 2- keto-gluconate and 5-keto-gluconate. Acid is weakly produced from D-tagatose and Turanose. The cell-wall peptidoglycan contains meso-diamino pimelic acid as diagnostic diamino acid. The major menaquinone is MK- 7. The polar lipid profile contains diphosphatidylglycerol, phosphatidylglycerol, a glycophospholipid, two glycolipids, three unidentified phospholipids and three unidentified lipids. The major fatty acids include iso-$C_{15:0}$, iso-$C_{17:0}$ and anteiso-$C_{17:0}$.

The type strain P1^T (=DSM 107529^T=CECT 9885^T=NCCB 100674^T) was isolated from a hot spring in Pasinler, Erzurum, Turkey. The genome of strain P1^T and its G+C content were estimated as 3.18 Mbp and 34.8%, respectively.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain P1^T is MG768952. The GenBank/EMBL/DDBJ accession number for the genome sequence of strain P1^T is JAACYS000000000. The version described in this paper is version JAACYS010000000.

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

Ethical statement
This article does not contain any studies with human participants and/or animals performed by any of the authors. The formal consent is not required in this study.

References

1. Cohn F. Untersuchungen uber Bakterien. *Beitr Biol Pflanz* 1872;1:127–224.
2. Poudel P, Miyamoto H, Miyamoto H, Okugawa Y, Tashiro Y *et al*. Thermotolerant *Bacillus kokeshiiformis* sp. nov. isolated from marine animal resources compost. *Int J Syst Evol Microbiol* 2014;64:2668–2674.
3. Dunlap CA, Schisler DA, Perry EB, Connor N, Cohan FM *et al*. *Bacillus swezeyi* sp. nov. and *Bacillus haynesii* sp. nov., isolated from desert soil. *Int J Syst Evol Microbiol* 2017;67:2720–2725.
4. Tidjani Alou M, Rathored J, Traore SI, Khelaifia S, Michelle C *et al*. *Bacillus niameyensis* sp. nov., a new bacterial species isolated from human gut. *New Microbes New Infect* 2015;8:61–69.
5. Menes RJ, Machin EV, Iriarte A, Langleib M. *Bacillus natronophilus* sp. nov., an alkaliphilic bacterium isolated from a soda lake. *Int J Syst Evol Microbiol* 2019.
6. Dong L, Wang S, Cao H, Zhao B, Zhang X *et al*. *Bacillus lacisalsi* sp. nov., a moderately haloalkaliphilic bacterium isolated from a saline-alkaline lake. *Antonie van Leeuwenhoek* 2020;113:127–136.
7. Liu Y, Yu M, Zhang X-H. *Bacillus alkalitolerans* sp. nov., isolated from marine sediment near a hydrothermal vent. *Int J Syst Evol Microbiol* 2018;68:1184–1189.
8. Shin B, Park C, Lee B-H, Lee K-E, Park W. *Bacillus miscanthi* sp. nov., a alkaliphilic bacterium from the rhizosphere of *Miscanthus sacchariflorus*. *Int J Syst Evol Microbiol* 2020;70:1843–1849.
9. Acevedo MM, Carroll LM, Mukherjee M, Mills E, Xlaoli L *et al*. *Bacillus clarus* sp. nov. is a new *Bacillus cereus* group species isolated from soil. *BioRxiv* 2019;508077.
10. Liu B, Liu G-H, Wang X-Y, Wang J-P, Chen Z *et al*. *Bacillus urbisdiaboli* sp. nov., isolated from soil sampled in Xinjiang. *Int J Syst Evol Microbiol* 2019;69:1591–1596.
11. Rao MPN, Dong Z-Y, Zhang H, Niu X-K, Zhang K *et al*. *Bacillus antri* sp. nov., isolated from cave soil. *Int J Syst Evol Microbiol* 2019;69:2335–2339.
12. Logan NA, Vos PD. Bacillus. Bergey's manual of systematics of archaea and bacteria 2015;17:1–63.
13. Albert RA, Archambault J, Rosselló-Mora R, Tindall BJ, Matheny M. *Bacillus acidicola* sp. nov., a novel mesophilic, acidophilic species isolated from acidic Sphagnum peat bogs in Wisconsin. *Int J Syst Evol Microbiol* 2005;55:2125–2130.
14. Olivera N, Siñeriz F, Breccia JD. *Bacillus patagoniensis* sp. nov., a novel alkalitolerant bacterium from the rhizosphere of *Atriplex lampa* in Patagonia, Argentina. *Int J Syst Evol Microbiol* 2005;55:443–447.
15. Ventosa A, García MT, Kamekura M, Onishi H, Ruiz-Berraquero F. *Bacillus halophilus* sp. nov., a moderately halophilic *Bacillus* species. *Syst Appl Microbiol* 1989;12:162–166.
16. 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.
17. Zarilla KA, Perry JJ. *Bacillus thermokovorans*, sp. nov., a species of obligately thermophilic hydrocarbon utilizing endospore-forming bacteria. *Syst Appl Microbiol* 1987;9:258–264.
18. Caccamo D, Gugliandolo C, Stackebrandt E, Maugeri TL. *Bacillus vulcani* sp. nov., a novel thermophilic species isolated from a shallow marine hydrothermal vent. *Int J Syst Evol Microbiol* 2000;50 Pt 6:2009–2012.
19. Sun Q-L, Yu C, Luan Z-D, Lian C, Hu Y-H *et al*. Description of *Bacillus kexueae* sp. nov. and *Bacillus manusensis* sp. nov., isolated from hydrothermal sediments. *Int J Syst Evol Microbiol* 2018;68:829–834.
20. Patel S, Gupta RS. A phylogenomic and comparative genomic framework for resolving the polyphyly of the genus *Bacillus*: Proposal for six new genera of *Bacillus* species, *Peribacillus* gen. nov., *Cytobacillus* gen. nov., *Mesobacillus* gen. nov., *Neobacillus* gen. nov., *Metabacillus* gen. nov. and *Alkalihalobacillus* gen. nov. *Int J Syst Evol Microbiol* 2020;70:406–438.
21. Maugeri TL, Gugliandolo C, Caccamo D, Panico A, Lama L *et al*. A halophilic thermotolerant *Bacillus* isolated from a marine hot spring able to produce a new exopolysaccharide. *Biotechnol Lett* 2002;24:515–519.
22. Irfan M, Tayyab A, Hasan F, Khan S, Badshah M *et al*. Production and Characterization of Organic Solvent-Tolerant Cellulase from *Bacillus amyloliquefaciens* AK9 Isolated from Hot Spring. *Appl Biochem Biotechnol* 2017;182:1390–1402.
23. Verma JP, Jaiswal DK, Krishna R, Prakash S, Yadav J *et al*. Characterization and Screening of Thermophilic *Bacillus* Strains for Developing Plant Growth Promoting Consortium From Hot Spring of Leh and Ladakh Region of India. *Front Microbiol* 2018;9:1293.
24. Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* 1991;173:697–703.
25. Yoon S-H, Ha S-M, Kwon S, Lim J, Kim Y *et al*. Introducing EzBioCloud: a taxonomically United database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 2017;67:1613–1617.
26. Edgar RC. Muscle: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 2004;32:1792–1797.
27. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4:406–425.
28. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* 2018;35:1547–1549.
29. Jukes TH, Cantor CR. Evolution of protein molecules. *Mammalian protein metabolism* 1969;3:132.
30. Meier-Kolthoff JP, Hahnke RL, Petersen J, Scheuner C, Michael V *et al*. Complete genome sequence of DSM 30083(T), the type strain (U5/41(T)) of *Escherichia coli*, and a proposal for delineating subspecies in microbial taxonomy. *Stand Genomic Sci* 2014;9:2.
31. Meier-Kolthoff JP, Göker M, Spröer C, Klenk H-P. When should a DDH experiment be mandatory in microbial taxonomy? *Arch Microbiol* 2013;195:413–418.
32. Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 2013;14:60.
33. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 2014;30:1312–1313.
34. Pattengale ND, Alipour M, Bininda-Emonds ORP, Moret BME, Stamatakis A. How many bootstrap replicates are necessary? *J Comput Biol* 2010;17:337–354.

35. Goloboff PA, Farris JS, Nixon KC. Tnt, a free program for phylogenetic analysis. *Cladistics* 2008;24:774–786.
36. Swofford DL. *Sinauer Associates*, 4. Sunderland, MA: 2002. PAUP*. Phylogenetic analysis using parsimony (* and other methods), version.; 2002. p. b10.
37. Stackebrandt E. Taxonomic parameters revisited: tarnished gold standards. *Microbiol Today* 2006;33:152–155.
38. Kim M, Oh H-S, Park S-C, Chun J. Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. *Int J Syst Evol Micr* 2014;64:1825.
39. Lee I, Chalita M, Ha S-M, Na S-I, Yoon S-H *et al*. ContEst16S: an algorithm that identifies contaminated prokaryotic genomes using 16S RNA gene sequences. *Int J Syst Evol Microbiol* 2017;67:2053–2057.
40. 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.
41. Blin K, Shaw S, Steinke K, Villebro R, Ziemert N *et al*. antiSMASH 5.0: updates to the secondary metabolite genome mining pipeline. *Nucleic Acids Res* 2019;47:W81–W87.
42. Kroppenstedt RM, Goodfellow M. The Family *Thermomonosporaceae: Actinocorallia, Actinomadura, Spirillospora* and *Thermomonospora*. *Prokaryotes: A Handbook on the Biology of Bacteria, Vol 3*, 3rd ed; 2006. pp. 682–724.
43. Garg N, Oman TJ, Andrew Wang T-S, De Gonzalo CVG, Walker S *et al*. Mode of action and structure-activity relationship studies of geobacillin I. *J Antibiot* 2014;67:133–136.
44. Gerhardt P, Murray RGE, Wood WA, Krieg NR. *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology; 1994.
45. Logan NA, Berge O, Bishop AH, Busse H-J, De Vos P *et al*. Proposed minimal standards for describing new taxa of aerobic, endospore-forming bacteria. *Int J Syst Evol Microbiol* 2009;59:2114–2121.
46. Sun L, Chen Y, Tian W, Yao L, Chen Z *et al*. *Bacillus acidinfaciens* sp. nov., isolated from farmland soil. *Int J Syst Evol Microbiol* 2019;69:1075–1080.
47. Schäffer C, Franck WL, Scheberl A, Kosma P, McDermott TR *et al*. Classification of isolates from locations in Austria and Yellowstone National Park as *Geobacillus tepidamans* sp. nov. *Int J Syst Evol Microbiol* 2004;54:2361–2368.
48. Habib N, Khan IU, Hussain F, Zhou E-M, Xiao M *et al*. *Meiothermus luteus* sp. nov., a slightly thermophilic bacterium isolated from a hot spring. *Int J Syst Evol Microbiol* 2017;67:2910–2914.
49. Lechevalier MP, Lechevalier H. Chemical composition as a criterion in the classification of aerobic *actinomycetes*. *Int J Syst Bacteriol* 1970;20:435–443.
50. Staneck JL, Roberts GD. Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* 1974;28:226–231.
51. Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M *et al*. An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Meth* 1984;2:233–241.
52. Collins MD. Analysis of isoprenoid quinones. *Methods Microbiol* 1985;18:329–366.
53. Sasser M. *Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids*, MIDI Technical Note 101. Newark: Midi Inc; 1990.
54. Coorevits A, Logan NA, Dinsdale AE, Halket G, Scheldeman P *et al*. *Bacillus thermolactis* sp. nov., isolated from dairy farms, and emended description of *Bacillus thermoamylovorans*. *Int J Syst Evol Microbiol* 2011;61:1954–1961.

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