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TAXONOMIC DESCRIPTION
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Genome-based classification of *Calidifontibacillus erzurumensis* gen. nov., sp. nov., isolated from a hot spring in Turkey, with reclassification of *Bacillus azotoformans* as *Calidifontibacillus azotoformans* comb. nov. and *Bacillus oryziterrae* as *Calidifontibacillus oryziterrae* comb. nov.

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Abstract

A novel Gram-stain-positive, rod-shaped, endospore-forming, motile, aerobic bacterium, designated as P2^T, was isolated from a hot spring water sample collected from Ilica-Erzurum, Turkey. Phylogenetic analyses based on 16S rRNA gene sequence comparisons affiliated strain P2^T with the genus *Bacillus*, and the strain showed the highest sequence identity to *Bacillus azotoformans* NBRC 15712^T (96.7%). However, the pairwise sequence comparisons of the 16S rRNA genes revealed that strain P2^T shared only 94.7% sequence identity with *Bacillus subtilis* subsp. *subtilis* NCIB 3610^T, indicating that strain P2^T might not be a member of the genus *Bacillus*. The digital DNA–DNA hybridization and average nucleotide identity values between strain P2^T and *B. azotoformans* NBRC 15712^T were 19.8 and 74.2%, respectively. The cell-wall peptidoglycan of strain P2^T contained *meso*-diaminopimelic acid. The polar lipid profile consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, an aminophospholipid, five unidentified phospholipids and two unidentified lipids while the predominant isoprenoid quinone was MK-7. The major fatty acids were iso-$C_{15:0}$ and iso-$C_{16:0}$. The draft genome of strain P2^T was composed of 82 contigs and found to be 3.5 Mb with 36.1 mol% G+C content. The results of phylogenomic and phenotypic analyses revealed that strain P2^T represents a novel genus in the family *Bacillaceae*, for which the name *Calidifontibacillus erzurumensis* gen. nov., sp. nov. is proposed. The type strain of *Calidifontibacillus erzurumensis* is P2^T (=CECT 9886^T=DSM 107530^T=NCCB 100675^T). Based on the results of the present study, it is also suggested that *Bacillus azotoformans* and *Bacillus oryziterrae* should be transferred to this novel genus as *Calidifontibacillus azotoformans* comb. nov. and *Calidifontibacillus oryziterrae* comb. nov., respectively.

The genus *Bacillus* consists of rod-shaped, endospore-forming and Gram-stain-positive bacteria that are included in the family *Bacillaceae* and was first proposed by Cohn in 1872 with the type species *Bacillus subtilis* [1]. Members of the genus are distributed ubiquitously in nature including in marine sediment [2], hydrothermal sediments [3], farmland soils [4], plant tissues [5] and hot springs [6]. This genus encompasses some well-known species such as *Bacillus anthracis*, *Bacillus cereus* and *Bacillus thuringiensis*, which have widespread impacts on human life. Members of the genus *Bacillus* exhibit extremely heterogenous phenotypic characteristics, as reflected by a polyphyletic nature in most phylogenetic trees [7, 8]. Thus, comprehensive taxonomic analyses based on reliable genomic markers should provide a rational basis for the resolution of the polyphyletic nature of the genus *Bacillus* as well as other genera in the family *Bacillaceae*.

Despite its importance for the assignment of a novel strain into a genus, the 16S rRNA gene is not enough to provide a clear evolutionary perception for larger taxa, as exemplified by recent phylogenomic works [8–10]. With the advent of next-generation sequencing platforms and the availability of many bacterial genomes, whole genome-based phylogenetic analyses have become the key to understanding evolutionary

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Abbreviation: RAST, rapid annotation using subsystem technology.
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain P2^T is MG777517. The GenBank/EMBL/DDBJ accession number for the genome sequence of strain P2^T is JABTTE000000000. The version described in this paper is version JABTTE000000000.
Three supplementary figures and four supplementary tables are available with the online version of this article.

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relationships within complex taxa. Therefore, phylogenomic approaches have been employed to resolve the issue of the phenotypic and phylogenetic heterogeneity of the genus *Bacillus* over the last decade by proposing novel genera or reclassification of existing strains into novel genera such as the creation of *Alkalihalobacillus*, *Cytobacillus*, *Mesobacillus*, *Metabacillus*, *Neobacillus* and *Peribacillus* for existing *Bacillus* strains [8] and the proposals of *Pradoshia* [11] and *Pueribacillus* [12] as novel genera in the family *Bacillaceae*.

During an investigation of thermophilic bacteria with potential industrial applications, the strain designated as P2^T was isolated from Ilica hot spring in Erzurum, Turkey, and its taxonomic position was determined by employing a polyphasic approach including whole genome-based analyses. Consequently, strain P2^T is considered as a type species of a novel genus in the family *Bacillaceae*, for which the name *Calidifontibacillus erzurumensis* gen. nov., sp. nov. is proposed. Based on phylogenomic analyses, we also propose the reclassification of *Bacillus azotoformans* and *Bacillus oryziterrae* as *Calidifontibacillus azotoformans* comb. nov. and *Calidifontibacillus oryziterrae* comb. nov., respectively.

ISOLATION AND ECOLOGY

Strain P2^T was isolated from water samples of Ilica hot spring (39° 56′ 44″ N, 41° 06′ 19″ E) in the province of Erzurum, Turkey. The temperature and pH of the hot spring were 39 °C and pH 7.0, respectively. The water samples were subjected to a filtering process within 2 h of sampling through Millipore membrane filters with a pore size of 0.45 µm. Microorganisms detained on the filters were transferred onto tryptic soy agar (TSA) and incubated aerobically at 55 °C for 48 h. The sample suspensions were then diluted 100-fold, and 100 µl samples was spread on TSA plates (peptone from casein, 15 g l^{-1}; peptone from soymeal, 5 g l^{-1}; NaCl, 5 g l^{-1}; agar, 15 g l^{-1}) and incubated at 55 °C for 48 h under aerobic conditions. Strain P2^T was selected and pure cultures were obtained after performing three successive transfers to fresh media. The strain was deposited on TSA plates at 4 °C and in 20% (v/v) glycerol stock solutions at −86 °C.

16S rRNA GENE PHYLOGENY

Genomic DNA extraction and purification were performed by using Wizard Genomic DNA Purification Kit (Promega). PCR amplification of the 16S rRNA gene was carried out using the universal primers 27F (5′-AGAGTTTGATCCTGGCTCA-3′) and 1492R (5′- GGTTACCTTGTTACGACTT-3′) [13]. The purified PCR product was ligated into pGEM-T Easy Vector Systems (Promega) and sequenced at Macrogen (Amsterdam, The Netherlands). An almost-complete 16S rRNA gene sequence (1509 bp) was obtained and deposited in the GenBank data library. Pairwise sequence analysis of the 16S rRNA gene was conducted in the EzBioCloud server (www.ezbiocloud.net; [14]) and the corresponding sequences of closely related type species of the family *Bacillaceae* were also retrieved from the same server. A multiple sequence alignment was generated using the MUSCLE algorithm [15] and a neighbour-joining tree [16] was reconstructed in MEGA X [17] using the model of Jukes and Cantor [18]. Moreover, phylogenetic analyses for the maximum-likelihood and maximum-parsimony methods were conducted by the GGDC web server using the DSMZ phylogenomics pipeline [19] adapted to single genes after pairwise sequence similarities were calculated according to Meier-Kolthoff *et al.* [20] via the GGDC web server (http://ggdc.ddsmz.de/) [21]. The maximum-likelihood tree was inferred from the alignment with RAxML [22] using rapid bootstrapping in conjunction with the autoMRE criterion [23] and the maximum-parsimony tree was inferred from the alignment with TNT [24] using 1000 bootstraps in conjunction with tree-bisection-and-reconnection branch swapping and ten additional random sequence replicates. The sequences were checked for compositional bias using the $\chi 2$ test as implemented in PAUP* [25].

Pairwise sequence analysis of the 16S rRNA gene of strain P2^T showed that the strain had high sequence identity with *Bacillus azotoformans* NBRC 15712^T (96.7%) while the gene identity level was lower than 96% for the rest of the genus *Bacillus*. However, the pairwise sequence identity level between strain P2^T and the type species of the genus *Bacillus* was calculated as 94.7%, which indicates that strain P2^T might not be a member of the genus *Bacillus*. Strain P2^T formed a distinct clade with the type strains of *Bacillus azotoformans* NBRC 15712^T and *Bacillus oryziterrae* ZYK^T in the phylogenetic trees based on maximum-likelihood (Fig. 1) and neighbour-joining algorithms (Fig. S1, available in the online version of this article).

GENOME FEATURES AND PHYLOGENOMIC ANALYSES

The whole genome of strain P2^T was sequenced using the Illumina HiSeq 2500 next generation sequencing platform with a 250 bp paired end protocol (MicrobesNG) and the draft genome sequence was submitted to the NCBI database under the accession number JABTTE000000000. The genome sequence was annotated on the Rapid Annotations using Subsystems Technology (RAST) server [26]. Predictions of secondary metabolite coding gene clusters and antibiotic resistance genes were achieved on the antiSMASH [27] and Antibiotic Resistance Target Seeker (ARTS) [28] web servers, respectively.

The draft genome is composed of 82 contigs with an N50 value of 113748. The numbers of coding sequences and RNAs are 3607 and 81, respectively. The total genome size is 3508867 bp and the G+C content is 36.1 mol%. The genome of strain P2^T encodes for a bacteriocin, a betalactone, a terpene and a type III polyketide synthase. The ARTS web server analysis showed that strain P2^T has antibiotic resistance genes, duplicated genes and evidence of horizontal gene transfer providing a strong basis for its biosynthetic potential. Strain P2^T has a total of 29 antibiotic resistance genes, 12 of which show close proximity to biosynthetic

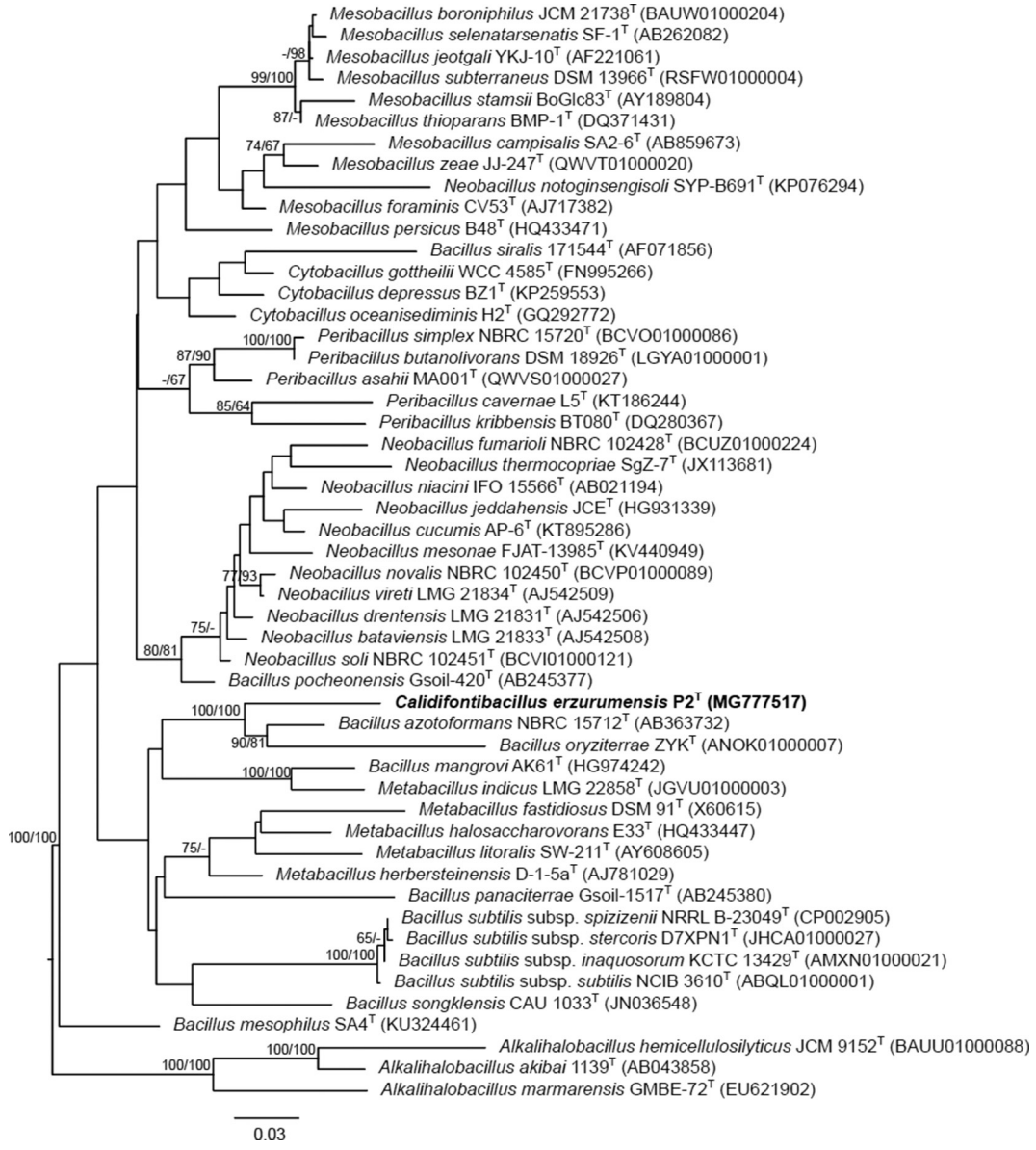


Fig. 1. Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences showing relationships between strain P2^T and the type strains of closely related *Bacillaceae* species. The tree was inferred under the GTR+CAT model and rooted by midpoint-rooting. The branches are scaled in terms of the expected number of substitutions per site. The numbers above the branches are support values when larger than 60% from maximum-likelihood (left) and maximum-parsimony (right) bootstrapping.

gene clusters. In addition, the number of duplicated genes and evidence of horizontal gene transfer were determined as 47 and 762, respectively. Strain P2^T has antimicrobial resistance genes encoding for antibiotic efflux pumps, class B beta-lactamase and quinolone resistance.

For a comprehensive analysis of the taxonomic position of the strain, a codon tree based on single-copy genes was reconstructed using amino acid and nucleotide sequences from PATRIC's global Protein Families (PGFams) [29]. The codon tree was generated based on the differences within the selected genome sequences of the family *Bacillaceae* (Table S1) after building an alignment on the PATRIC server (https://patricbrc.org/). Briefly, both protein and gene sequences were used for each of the selected genes from the PGFams. Protein sequences were aligned using MUSCLE [15] and gene sequences were aligned using the codon align function of BioPython [30]. A concatenated alignment of all proteins and nucleotides was written to a phylip formatted file, and then a partitions file for RAxML [22] was generated, describing the alignment in terms of proteins and then codon positions. Support values were generated using 100 rounds of the rapid bootstrapping option [31] of RAxML.

For reconstruction of a codon tree, a total of 198 single-copy genes (Table S2) was used. In the codon tree, the

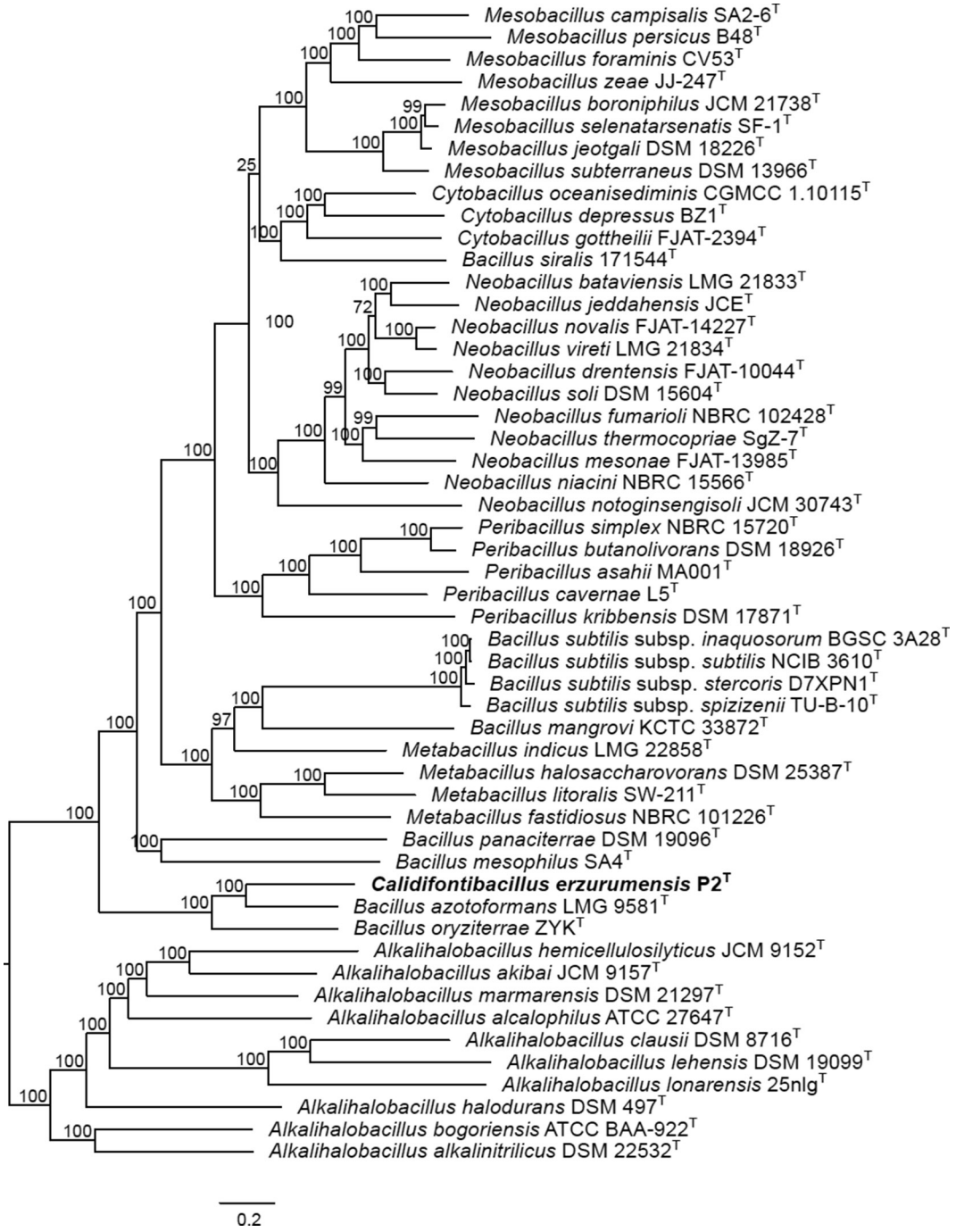


Fig. 2. The codon tree of the members of the family *Bacillaceae* built using the PATRIC server (*https://patricbrc.org/app/PhylogeneticTree*). A total of 198 single-copy genes found for 52 genomes and both amino acid and nucleotide sequences were used for each gene. The number of amino acids aligned using MUSCLE [15] was 50636 and the number of nucleotides aligned using the codon_align function of BioPython [30] was 150927.

recently described genera by Patel and Gupta [8] as well as the remaining species in the genus *Bacillus* formed well-supported clusters (Fig. 2) . Strain P2^T was clustered together with *Bacillus azotoformans* LMG 9581^T and *Bacillus oryziterrae* ZYK^T by forming a separate clade clearly distinguishable from other genera in the family *Bacillaceae*. Moreover, each of the recently described genera *Alkalihalobacillus*, *Cytobacillus*, *Mesobacillus*, *Neobacillus* and *Peribacillus* formed monophyletic clades while the genus *Metabacillus* was still paraphyletic with the members of the genus *Bacillus*. In a recent revision of the genus *Bacillus*, several molecular markers comprising conserved signature

Table 1. Differential phenotypic characteristics between strain P2^T and phylogenetically related species

Strains: 1, P2^T; 2, *Bacillus azotoformans* ATCC 29788^T. 3, *Bacillus oryziterrae* DSM 26460^T +, Positive; w, weakly positive; –, negative; ND, not detected. All experiments were performed under identical conditions unless otherwise stated. All strains are positive for motility and growth in the presence of 1% NaCl while negative for catalase activity, aesculin hydrolysis, acid production from D-mannitol or D-xylose, and growth in the presence of 8% NaCl and at pH 4.5.

Characteristic	1	2	3*
Oxidase activity	+	+	–
Oxygen Requirements	Aerobic	Facultatively anaerobic	Facultatively anaerobic
Hydrolysis of:			
Starch	–	+	–
Casein	–	+	–
Gelatine	+	–	–
Acid production from:			
Ribose	+	–	ND
D-Glucose	+	–	+
D-Fructose	+	–	ND
Maltose	+	–	+
Trehalose	+	–	ND
Melezitose	W	–	ND
D-Mannose	–	+	–
Growth with NaCl at:			
3 %	+	+	–
5 %	–	+	–
Growth at pH 9	+	–	–
Growth at temperature:			
30 °C	–	+	+
60 °C	+	–	–
DNA G+C content (mol%)	36.1	37.2	36.1
Isolation source	Hot spring	Soil*	Soil

*Data taken from Bao *et al.* [54]

indels in protein sequences were used to differentiate the above-mentioned genera while *Bacillus azotoformans* and *Bacillus oryziterrae* could not be grouped into the 'Subtilis' or 'Cereus' clades or into newly differentiated clades [8]. Moreover, both species formed distinct clusters together with strain P2^T in phylogenetic trees based on multiple sequence alignment analyses of DNA gyrase subunit A and B, DNA topoisomerase I and RNA polymerase sigma factor RpoD protein sequences constructed on the PATRIC server using the protein family sorter tool [32].

The calculation of digital DNA–DNA hybridization (dDDH) and average nucleotide identity (ANI) values were performed on the GGDC (https://ggdc.dsmz.de/) [20] and JSpecies (http://jspecies.ribohost.com/jspeciesws/#home) [33] web servers, respectively. The dDDH value between strain P2^T and its close phylogenetic neighbour *B. azotoformans* LMG 9581^T was 19.8% while that with *B. oryziterrae* ZYK^T was 19.2%. The ANI levels of strain P2^T with *B. azotoformans* LMG 9581^T and *B. oryziterrae* ZYK^T were calculated as 74.2 and 72.5%, respectively. Both dDDH and ANI values are well below the thresholds to differentiate the strain as a novel species. On the other hand, the ANI level between strain P2^T and the type species of the genus *Bacillus* was determined as 67% indicating its distant affiliation to the genus *Bacillus* (Table S3).

In order to clarify the taxonomic position of strain P2^T, genome-based comparisons for conserved protein-coding genes were performed by calculating average amino acid identity (AAI) levels [34] between strain P2^T and its close neighbours using the AAI matrix calculator available at http://enve-omics.ce.gatech.edu/g-matrix/ [35]. AAI values between strain P2^T and its closest neighbours *B. azotoformans* LMG 9581^T and *B. oryziterrae* ZYK^T were calculated as 74and 70%, respectively, while AAI values were lower than 60% for the type species of newly described genera in the family *Bacillaceae* as well as for the type species of the genus *Bacillus* (Table S4). On the other hand, the AAI value between *B. azotoformans* LMG 9581^T and *B. oryziterrae* ZYK^T was determined as 71%. Considering recent works on genus delineation based on mean protein sequence similarity of all protein-coding genes [36–38], members of the family *Bacillaceae* can be distinguished by 65–70% AAI value at genus level. Consequently, strain P2^T together with its close phylogenetic neighbours, i.e. *B. azotoformans* LMG 9581^T and *B. oryziterrae* ZYK^T, are considered to represent a novel genus within the family *Bacillaceae*.

PHYSIOLOGY AND CHEMOTAXONOMY

According to the minimal standards to describe new taxa of aerobic and endospore-forming bacteria [39], phenotypic characterization of strain P2^T was carried out.

Gram-staining of cells was carried out by using the method of Gerhardt *et al.* [40]. Motility was checked by observing dispersed growth of cells in test tubes containing semi-solid TSA agar (0.3%, w/v) and photomicrograph of cells with flagella was taken by TEM (Fig. S2a) [4]. Spore characteristics were observed by light microscopy after malachite green staining and transmission electron microscopy (Fig. S2b, c) [39]. Growth characteristics under anaerobic conditions were tested as described by Schäffer [41].

To determine optimal conditions for strain P2^T, cells were grown in tryptic soy broth (TSB) medium at different temperatures (25, 30, 35, 40, 45, 50, 55, 60, 65 and 70 °C)

Table 2. General characteristics of strain P2^T and closely related genera in the family *Bacillaceae*

Characteristic	P2^T	*Alkalihalobacillus**, †	*Bacillus**, †	*Cytobacillus**, †	*Mesobacillus**, †	*Metabacillus**, †	*Neobacillus**, †	*Peribacillus**, †
Endospore	+	+	+	+	+	+	+	+
Cell-wall amino acid	*meso*-DAP	*meso*-DAP	*meso*-DAP	*meso*-DAP	*meso*-DAP	*meso*-DAP	*meso*-DAP	*meso*-DAP
Polar lipids	DPG, PG, PE, APL, PL, L	DPG, PE, PG	DPG, PE, PG	DPG, PE, PG	DPG, PE, PG	DPG, PE, PG	DPG, PE, PG	DPG, PE, PG
Quinone	MK-7	MK-7	MK-7	MK-7	MK-7	MK-7	MK-7	MK-7
Fatty acids	iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{14:0}$, $C_{16:0}$	iso-$C_{15:0}$, anteiso-$C_{15:0}$, iso-$C_{14:0}$	iso-$C_{15:0}$, anteiso-$C_{15:0}$	iso-$C_{15:0}$, anteiso-$C_{15:0}$, iso-$C_{14:0}$	iso-$C_{15:0}$, anteiso-$C_{15:0}$	iso-$C_{15:0}$, anteiso-$C_{15:0}$	iso-$C_{15:0}$, anteiso-$C_{15:0}$	anteiso-$C_{15:0}$, iso-$C_{15:0}$
Genome size (Mb)	3.5	2.5–5.5	3.5–5.4	4.5–5.6	4.2–5.7	4.1–5.3	3.2–6.1	4.3–5.6
DNA G+C content (mol%)	36.1	36.7–44.6	36.1–46.6	38.1–40.8	33.7–45.4	35.1–44.4	37.3–43.7	37.4–43.0

*Data taken from Logan and De Vos [50].
†Data taken from Patel and Gupta [8].

for up to 72 h. The pH ranges for growth were tested in TSB at pH 3.0–11.0 (0.5 pH unit intervals), using appropriate biological buffers: citrate/phosphate (pH 3.0–7.0); Tris/HCl (pH 8.0–9.0); or sodium carbonate/sodium bicarbonate (pH 10.0–11.0) [42]. Growth tolerance to NaCl was determined using TSB supplemented with 0–10% NaCl (at intervals of 1.0%) for 72 h at 45 °C. All parameters (temperature, salt concentration and pH value) were tested in triplicate and growth was measured at OD_{600} nm. Oxidase reagent (Sigma) was used for testing oxidase activity. Catalase activity was observed by the production of bubbles after the addition of a drop of 3% H_2O_2 (v/v). The ability of strain P2^T to grow on various media including TSA (pancreatic digest of casein, 15.0 g l^{-1}; peptic digest of soybean meal, 5.0 g l^{-1}; sodium chloride, 5.0 g l^{-1}; agar, 15.0 g l^{-1}), nutrient agar (peptone, 5.0 g l^{-1}; yeast extract, 2.0 g l^{-1}; sodium chloride, 5.0 g l^{-1}; agar, 15.0 g l^{-1}), Luria–Bertani (LB) agar (tryptone, 10.0 g l^{-1}; yeast extract, 5.0 g l^{-1}; NaCl, 10.0 g l^{-1}; agar, 15.0 g l^{-1}) and plate count agar (enzymatic digest of casein/tryptone, 5.0 g l^{-1}; yeast extract, 2.5 g l^{-1}; glucose, 1.0 g l^{-1}; agar, 15.0 g l^{-1}) was analysed. Hydrolyses of casein and starch were performed on modified TSA supplemented with soluble casein (1%, w/v) or starch (1%, w/v), respectively. Biochemical tests were conducted using API 20E, API 50CH/B and API ZYM strips according to the manufacturer's instructions (bioMérieux). Arginine hydrolase, ornithine decarboxylase, Voges–Proskauer test, production of indole, hydrolysis of aesculin and *p*-nitrophenyl-β-D-galactopyranoside (pNPG) and the assimilation of carbon sources were tested with the API 20E system (bioMérieux) as recommended by the manufacturer. Nitrate reductase, urease and gelatinase activities were also designated using the API 20E system. Production of other enzymes was determined using the API ZYM syste. Acid production from different carbohydrates was investigated using the API 50CH system. 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 [43]. All API tests were performed in triplicate.

Biomass for chemotaxonomic analyses was obtained by cultivation of the strain in shake flasks at 45 °C for 3 days on a rotary shaker (150 r.p.m.) using 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 [44] and analysed by TLC according to Staneck and Roberts [45]. Polar lipids were extracted and analysed by two-dimensional TLC following Minnikin *et al.* [46] using modifications of Kroppenstedt and Goodfellow [47]. Menaquinones were extracted from freeze-dried biomass and purified according to Collins [48]. The extracted menaquinones were analysed using HPLC (LC-10AS, Shimadzu) fitted with a reverse-phase C18 column (150×4.6 mm, 5 µm particle size; RP-18-Lichrosorb, Penomenex) at 270 nm. Fatty acid methyl esters were extracted from fresh cultures grown on TSA at 45 °C for 24 h and analysed using the Microbial Identification System (MIDI) according to the technical instructions provided by this system [49].

Cells of strain P2^T were Gram-stain-positive, motile and spore-forming. Strain P2^T grew at pH values ranging from pH 6 to 10 and at 40–60 °C while optimum growth was observed at pH 8 and 45 °C. Strain P2^T could tolerate NaCl up to 4% (w/v) and could grow optimally in the presence of 2% NaCl. The strain was positive for the oxidase test, but negative for the catalase test. Strain P2^T could not grow under anaerobic conditions. The strain was able to grow

Table 3. Fatty acid profiles of strain P2^T and its phylogenetic neighbours

The strains were grown under identical conditions unless otherwise stated. Major components are indicated with bold text. –, Not detected.

Farry acid	Strain P2^T	*Bacillus azotoformans* ATCC 29788^T	*Bacillus oryziterrae* DSM 26460^{T*}
Saturated:			
$C_{9:0}$	–	–	1.9
$C_{14:0}$	3.4	2.6	4.7
$C_{16:0}$	10.3	1.7	**32**
$C_{17:0}$	3.5	–	–
$C_{18:0}$	3.4	0.9	4.9
$C_{20:0}$	–	–	–
Unsaturated:			
$C_{16:1}$ $\omega 7c$	–	1.3	–
$C_{18:0}$ 10-methyl	–	–	–
$C_{17:1}$ $\omega 9c$	–	–	–
Branched:			
iso-$C_{13:0}$	–	2.8	–
iso-$C_{14:0}$	11.3	**20.7**	**17.8**
iso-$C_{15:0}$	**41.1**	**49.8**	**17.8**
iso-$C_{15:1}$ $\omega 9c$	–	2	–
iso-$C_{16:0}$	**24.9**	1.1	10.5
iso-$C_{17:0}$	5.6	–	–
iso-$C_{16:1}$ $\omega 11c$	–	1.9	–
anteiso-$C_{15:0}$	3.5	**15.1**	10.4
anteiso-$C_{17:0}$	**12.5**	–	–

*Data taken from Bao *et al*. [54]

well on TSA medium but not on LB agar, nutrient agar and plate count agar media. Strain P2^T could not hydrolyse starch, casein or aesculin. Positive results were obtained for acid production from ribose, D-glucose, D-fructose, maltose and trehalose. Detailed physiological characteristics of the strain are given in Table 1 and the species description.

Chemotaxonomic characteristics of strain P2^T were consistent with those of members of the family *Bacillaceae* (Table 2). The peptidoglycan of strain P2^T contained *meso*-DAP as the cell-wall diamino acid. The polar lipid profile was found to contain diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, an aminophospholipid, five unidentified phospholipids and two unidentified lipids (Fig. S3). The predominant menaquinone was determined to be MK-7 (97%). The major cellular fatty acids were identified as iso-$C_{15:0}$ (41.1%), iso-$C_{16:0}$ (24.9%), iso-$C_{14:0}$ (11.2%) and $C_{16:0}$ (10.2%) (Table 3). Although members of the family *Bacillaceae* have largely common chemotaxonomical characteristics, strain P2^T and its phylogenetic neighbours *B. azotoformans* and *B. oryziterrae* differed from closely related genera by the presence of aminophospholipid in their polar lipid profiles.

A comparative polyphasic analysis between strain P2^T and closely related type strain *B. azotoformans* NBRC 15712^T revealed that the strain could be distinguished from the closely related type species on the basis of chemical, molecular and physiological characteristics, including acid production from ribose, glucose, fructose, maltose and trehalose as well as growth at 60 °C and pH 9.0. However, both strain P2^T and *B. azotoformans* together with *B. oryziterrae* have common characteristics such as polar lipid profile and similar DNA G+C content ranging from 36 to 37 mol%. In addition, strain P2^T and its close phylogenetic neighbours *B. azotoformans* and *B. oryziterrae* formed distinct clades in all reconstructed trees, i.e. the codon tree based on 198 single-copy genes and the 16S rRNA gene trees based on the neighbour-joining, maximum-likelihood and maximum-parsimony algorithms. In a recent revision of the family *Bacillaceae* based on conserved signature indels [8], both *B. azotoformans* and *B. oryziterrae* could not be grouped into newly described genera in the family *Bacillaceae*. Moreover, low levels of overall genome relatedness indices of AAI, ANI and dDDH between strain P2^T together with its close phylogenetic neighbours and the type strain of the genus *Bacillus* provided a strong basis for classification of strain P2^T as a representative of novel genus. As stated in previous works [8, 50–52], the genus *Bacillus* represents a large assemblage of phylogenetically unrelated micro-organisms and a thorough reclassification scheme should be provided in order to solve the extreme polyphyly of the species within the genus. Consistent with this idea, we describe strain P2^T as a type species of a novel genus in the family *Bacillaceae*, for which the name *Calidifontibacillus erzurumensis* gen. nov., sp. nov. is proposed. In addition, its close phylogenetic neighbours *B. azotoformans* and *B. oryziterrae* are reclassified in this novel genus as *Calidifontibacillus azotoformans* comb. nov. and *Calidifontibacillus oryziterrae* comb. nov., respectively.

DESCRIPTION OF *CALIDIFONTIBACILLUS* GEN. NOV.

Calidifontibacillus (Ca.li.di.fon.ti.ba.cil'lus. L. adj. *calidus*, hot; L. masc. n. *fons*, fontis spring; L. masc. n. *bacillus* a small staff or rod and *Bacillus*, a bacterial genus; N.L. masc. n. *Calidifontibacillus*, a rod isolated from hot spring)

Cells are Gram-stain-positive, short-rod-shaped, motile, endospore-forming and catalase-negative. The major isoprenoid quinone is MK-7. The polar lipid profile contains diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, aminophospholipid and unidentified phospholipids. The major fatty acids are iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{14:0,}$ $C_{16:0}$ and anteiso-$C_{15:0}$. The DNA G+C content

is 36–37 mol%. The type species is *Calidifontibacillus erzurumensis*.

DESCRIPTION OF *CALIDIFONTIBACILLUS ERZURUMENSIS* SP. NOV.

Calidifontibacillus erzurumensis (er.zu.rum.en′sis. N.L. masc. adj. *erzurumensis* of Erzurum, where the type strain was isolated).

Cells are Gram-stain-positive, aerobic, rod-shaped, motile, endospore-forming and approximately 0.4–0.7 µm in diameter and 2.5–6.0 µm long. Colonies are clear-coloured, filamentous, raised and irregular in shape. Growth on TSA medium occurs at pH 6.0–10.0 (optimum, pH 8.0), at 40–60 °C (optimum, 45 °C) and in the presence of 0–4% (w/v) NaCl (optimum, 2% NaCl). Aesculin, casein, urea and starch are not hydrolysed. Ribose, D-glucose, D-fructose, maltose and trehalose are utilized. Citrate, mannose, *N*-acetyl-D-glucosamine, phenylacetic acid and capric acid are not utilized. Naphthol-AS-BI-phosphohydrolase activity is present while O-nitrophenyl-β-D-galactopyranoside, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are absent. Positive for the Voges–Proskauer test but negative for H_2S and indole production. In the API 50CHB gallery, high quantities of acids are produced from ribose, D-glucose, D-fructose, maltose and trehalose. Acid is not produced from glycerol, erythritol, D-arabinose, L-arabinose, D-xylose, L-xylose, adonithol, methyl xyloside, galactose, D-mannose, sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl D-mannoside, methyl D-glucoside, *N*-acetyl glucosamine, amygdalin, arbutin, aesculin, salicin, cellobiose, lactose, melibiose, sucrose, inulin, raffinose, starch, glycogen, xylitol, gentibiose, turanose, lyxose, tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate or 5-ketogluconate. The cell-wall peptidoglycan contains *meso*-diaminopimelic acid as the diagnostic diamino acid. The major menaquinone is MK-7. The polar lipid profile contains diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, an aminophospholipid, five unidentified phospholipids and two unidentified lipids. The major fatty acids include iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{14:0}$ and $C_{16:0}$.

The type strain, P2^T (=CECT 9886^T=DSM 107530^T=NCCB 100675^T), was isolated from a hot spring in Ilica, Erzurum, Turkey. The genome of strain P2^T and its G+C content were estimated as 3.5 Mbp and 36.1 mol%, respectively.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain P2^T is MG777517. The GenBank/EMBL/DDBJ accession number for the genome sequence of strain P2^T is JABTTE000000000. The version described in this paper is version JABTTE000000000.

DESCRIPTION OF *CALIDIFONTIBACILLUS AZOTOFORMANS* COMB. NOV.

Calidifontibacillus azotoformans (a.zo.to.for′mans French n. *azote*, nitrogen; N.L. n. *azotum*, nitrogen; L. part. adj. *formans*, forming; N.L. part. adj. *azotoformans*, nitrogen-forming).

Basonym: *Bacillus azotoformans* Pichinoty *et al.* 1983 [53].

The description is as before [54] with the following addition. The DNA G+C content of the type strain genome is 37.2 mol%, its approximate size 4.2 Mb, its GenBank accession number is AJLR00000000.

The type strain of *Calidifontibacillus azotoformans* is ATCC 29788^T (=CCM 2849^T=CCUG 28517^T=CIP 77.28^T=CIP R925^T=DSM 1046^T=HAMBI 1884^T=IFO 15712^T=JCM 12210^T=LMG 9581^T=NBRC 15712^T=NRRL B-14310^T=NRRL B-14522^T).

DESCRIPTION OF *CALIDIFONTIBACILLUS ORYZITERRAE* COMB. NOV.

Calidibacillus oryziterrae (o.ry.zi.ter′rae. L. n. *oryza*, rice; L. n. *terra*, earth, soil; N. L. gen. n. *oryziterrae*, of the rice soil or field, referring to the source of the type strain).

Basonym: *Bacillus oryziterrae* Bao *et al.* 2017 [54].

The description is identical to that given for *B. oryziterrae* by Bao *et al.* [54]. The genome size of the type strain is approximately 3.5 Mb and the GenBank accession number is ANOK00000000. The type strain of *Calidifontibacillus oryziterrae* is ZYK^T (=CGMCC 1.5179^T=DSM 26460^T).

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

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