Anoxybacillus vitaminiphilus sp. nov., a strictly aerobic and moderately thermophilic bacterium isolated from a hot spring

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A strictly aerobic, Gram-stain-positive, motile and spore-forming bacterium, strain 3nP4^T, was isolated from the Puge hot spring located in the south-western geothermal area of China. Strain 3nP4^T grew at 38–66 °C (optimum 57–60 °C), at pH 6.0–9.3 (optimum 7.0–7.5) and with 0–4 % (w/v) NaCl (optimum 0–0.5 %). Phylogenetic analysis of 16S rRNA gene sequences, as well as DNA–DNA relatedness values, indicated that the isolate represents a novel species of the genus *Anoxybacillus*, related most closely to *Anoxybacillus voinovskiensis* DSM 12111^T. Strain 3nP4^T had diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and one unidentified phospholipid as major polar lipids and iso-$C_{15:0}$ and iso-$C_{17:0}$ as major fatty acids, which are both typical chemotaxonomic characteristics of the genus *Anoxybacillus*. The mean DNA G+C content of strain 3nP4^T was 39.2 ± 0.95 mol% (HPLC). A distinctive characteristic of the novel isolate was its extreme reliance on vitamin mixture or yeast extract for growth. Based on data from this taxonomic study using a polyphasic approach, strain 3nP4^T is considered to represent a novel species of the genus *Anoxybacillus*, for which the name *Anoxybacillus vitaminiphilus* sp. nov. is proposed. The type strain is 3nP4^T (=CGMCC 1.8979^T=JCM 16594^T).

According to the List of Prokaryotic names with Standing in Nomenclature, the genus *Anoxybacillus* comprises, at the time of writing, 18 recognized species: *Anoxybacillus pushchinoensis* and *Anoxybacillus flavithermus* (Pikuta *et al.*, 2000), *Anoxybacillus gonensis* (Belduz *et al.*, 2003), *Anoxybacillus contaminans* (De Clerck *et al.*, 2004), *Anoxybacillus voinovskiensis* (Yumoto *et al.*, 2004), *Anoxybacillus ayderensis* and *Anoxybacillus kestanbolensis* (Dulger *et al.*, 2004), *Anoxybacillus kamchatkensis* (Kevbrin *et al.*, 2005), *Anoxybacillus amylolyticus* (Poli *et al.*, 2006), *Anoxybacillus rupiensis* (Derekova *et al.*, 2007), *Anoxybacillus bogrovensis* (Atanassova *et al.*, 2008), *Anoxybacillus tengchongensis* and *Anoxybacillus eryuanensis* (Zhang *et al.*, 2011), *Anoxybacillus salavatliensis* (Cihan *et al.*, 2011), *Anoxybacillus mongoliensis* (Namsaraev *et al.*, 2010), *Anoxybacillus thermarum* (Poli *et al.*, 2009), *Anoxybacillus tepidamans* and *Anoxybacillus caldiproteolyticus* (Coorevits *et al.*, 2012), listed in their order of validation. A novel subspecies of *Anoxybacillus flavithermus*, *Anoxybacillus flavithermus* subsp. *yunnanensis*, was described by Dai *et al.* (2011).

The genus *Anoxybacillus* was initially characterized by anaerobic growth (Pikuta *et al.*, 2000). According to the emended description of this genus (Pikuta *et al.*, 2003), most species of the genus *Anoxybacillus* are facultative anaerobes and several are facultative aerobes which only grow under certain anaerobic conditions (Yumoto *et al.*, 2004; Kevbrin *et al.*, 2005). Three strictly aerobic species, *Anoxybacillus rupiensis*, *Anoxybacillus thermarum* and *Anoxybacillus caldiproteolyticus*, have also been described, which greatly expands the phenotypic diversity of this genus. In this paper, a novel strictly aerobic species of the genus *Anoxybacillus* is proposed based on the description of a novel strain, 3nP4^T, which was isolated from a hot spring in the south-western geothermal area of China.

A water sample was collected from the Puge hot spring in Puge county (27° 13′–27° 30′ N 102° 26′–102° 46′ E), Sichuan province, China. Temperature and pH *in situ* were 42–44 °C and pH 7. The sample was transported without temperature control and stored at 4 °C in the lab until used. Reference strains used in this paper were *Aeribacillus pallidus* DSM 3670^T, *Anoxybacillus voinovskiensis* DSM 12111^T, *Anoxybacillus rupiensis* DSM 17127^T, *Anoxybacillus tepidamans* DSM 16325^T, *Anoxybacillus amylolyticus* DSM

Abbreviations: DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; RAPD, random amplified polymorphic DNA.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Anoxybacillus vitaminiphilus* strain 3nP4^T is FJ474084.

A supplementary table and four supplementary figures are available with the online version of this paper.

15939^T and *Anoxybacillus contaminans* DSM 15866^T. Unless otherwise indicated, Brock's basal salts (Brock *et al.*, 1972) supplemented with NaCl (5.0 g l^{-1}) was used as the basic mineral medium. Modified Brock's medium consisting of tryptone (3.0 g l^{-1}; Difco), yeast extract (3.0 g l^{-1}; Difco) and the basic mineral medium was used for cultivation. With the exception of *Anoxybacillus contaminans* DSM 15866^T, which was cultured at 50 °C, all other strains were incubated at 57 °C.

For the enrichment culture, 200 ml spring water was filtered through the filter (0.22 μm). The membrane was subsequently inoculated into 20 ml sterile spring water supplemented with tryptone (1.0 g l^{-1}) and yeast extract (1.0 g l^{-1}). Parallel preparations were cultivated at 60 °C and 120 r.p.m. in a rotary water-bath shaker. The turbid cultures were serially diluted and spread onto agar plates. All plates were incubated at 50 °C until visible colonies formed. Distinctive colonies were picked out and sub-cultured at least three times for purification and then preserved by freeze-drying.

Cells in exponential phase of growth were used for characterization of motility and cell morphology. Observation was performed by optical microscopy (BX40; Olympus) and transmission electron microscopy (JEM-1230; JEOL). Temperature range for growth was tested in 2 °C steps until no growth was observed after incubation for 7 days. The effect of pH was determined in 0.5 pH steps using 30 mM buffering agents, including MES (pH 5.0–6.5), MOPS (pH 6.5–8.0), Tricine (pH 8.0–9.0) and CAPSO (pH 9.0–10.5). Salt tolerance was examined by increasing NaCl concentrations up to 5 % (w/v).

The assimilation of single carbon sources was tested in the basic mineral medium supplemented with 0.01 % (w/v) yeast extract and 0.1 % (w/v) of the carbon source. The assimilation of single nitrogen sources was also tested in the basic mineral medium except that $(NH_4)_2SO_4$ was substituted by 0.1 % (w/v) of the nitrogen source and additional 0.1 % (w/v) glucose. Vitamin mixture (Balch *et al.*, 1979) was required for growth of strain 3nP4^T in all assimilation tests. Other physiological and biochemical characteristics were examined as described previously (Yumoto *et al.*, 2004; Poli *et al.*, 2006; Derekova *et al.*, 2007). Similarly, the vitamin mixture was required for growth of strain 3nP4^T unless yeast extract was added. All tested strains were incubated for 7 days.

The requirement of vitamin mixture or yeast extract for growth was checked in basic mineral medium containing certain proteinaceous substrates (1.0 g l^{-1}; Difco) including tryptone, peptone or Casamino acids. For control, a culture without supplement was incubated under the same conditions. All reference strains described above were used for comparison.

Sensitivity to antibiotics was assayed with a two-layer plate method as described previously (Zhang *et al.*, 2010) except that modified Brock's medium was used. All plates were cultivated for 4 h before the antibiotic discs were placed and then re-incubated overnight for observation.

Cultures used for polar lipid, isoprenoid quinone and fatty acid analyses were all incubated in 250 ml flasks containing 100 ml modified Brock's medium in rotary water-bath shakers at 100 r.p.m. until the exponential phase. Polar lipids were extracted as described by Kates (1986). Extracts were separated by two-dimensional TLC on silica gel 60 F254 plates (Merck) and sprayed with sulfuric acid/ethanol to detect total polar lipids as specified by Cui *et al.* (2011). Other specific reagents were also used for the detection of sugars, phosphate and free amino groups as described by Tindall (1990). Isoprenoid quinones were extracted from freeze-dried cells and purified according to Komagata & Suzuki (1987). Preparations were analysed using an LC-MS system (Agilent). For fatty acid analysis, fatty acid methyl esters were obtained and analysed as described previously (Zhang *et al.*, 2010).

A quick bacteria genomic DNA extraction kit (DongSheng Biotech) was used for template preparation. The 16S rRNA gene was amplified with primer pair 27F/1492R and cloned into pMD 19-T vector (TaKaRa) for sequencing. Sequence similarity was calculated using the EzTaxon-e server (Kim *et al.*, 2012). All related sequences were exported to the MEGA5 software package (Tamura *et al.*, 2011) for multiple sequence alignment and phylogenetic tree reconstruction. Trees were reconstructed using the neighbour-joining and maximum-likelihood methods. Evolutionary distances used in the neighbour-joining method were calculated according to the algorithm of the Kimura two-parameter model (Kimura, 1980).

The G+C content of the genomic DNA of strain 3nP4^T was determined by HPLC (Mesbah *et al.*, 1989) and by the thermal denaturation temperature (T_m) method (Marmur & Doty, 1962) with *Escherichia coli* K-12 as a calibration standard. DNA–DNA hybridization experiments were performed as specified previously (Zhang *et al.*, 2010).

PCR fingerprinting analysis, including random amplified polymorphic DNA (RAPD) and internal transcribed spacer polymorphism, was performed according to Ronimus *et al.* (1997) and Kuisiene *et al.* (2002). Amplification was repeated at least three times to check the polymorphic reproducibility.

Twelve strains were isolated from the water sample of the Puge hot spring. All were moderately thermophilic and spore-forming bacteria. On the basis of phylogenetic analysis, 11 strains were closely related to typical thermophilic bacilli, namely *Geobacillus stearothermophilus*, *Geobacillus thermoleovorans*, *Geobacillus kaustophilus*, *Geobacillus subterraneus*, *Anoxybacillus pushchinoensis* and *Brevibacillus thermoruber*, sharing 99–100 % 16S rRNA gene sequence similarity with their type strains. However, strain 3nP4^T had 97.08 % sequence similarity with *Anoxybacillus voinovskiensis* DSM 12111^T, and therefore was considered to represent a novel species of the genus *Anoxybacillus* and selected for further taxonomic research.

Table 1. Phenotypic and genotypic characteristics of strain 3nP4^T and the type strains of closely related species

Strains: 1, 3nP4^T; 2, *Aeribacillus pallidus* DSM 3670^T; 3, *Anoxybacillus voinovskiensis* DSM 12111^T; 4, *Anoxybacillus rupiensis* DSM 17127^T; 5. *Anoxybacillus tepidamans* DSM 16325^T; 6. *Anoxybacillus amylolyticus* DSM 15939^T; 7. *Anoxybacillus contaminans* DSM 15866^T. All data were from the present study. All strains were negative for citrate utilization, indole production, phenylalanine deamination, tyrosine degradation, hydrolysis of Tweens 20 and 80, and utilization of L-sorbose, dulcitol, erythritol, formate, L-methionine and L-serine. All strains were positive for catalase activity, hydrolysis of Tweens 40 and 60, utilization of D-fructose, D-glucose, maltose, sucrose, trehalose, acetate, pyruvate, L-asparagine, L-aspartic acid, L-glutamate and L-glutamine, acid production from D-fructose, D-glucose, maltose, sucrose and trehalose, and assimilation of casamino acids, peptone, tryptone, yeast extract, urea and ammonium sulfate as single nitrogen source. Sensitive to ampicillin (10 μg), carbenicillin (100 μg), chloramphenicol (30 μg), erythromycin (15 μg), gentamicin (10 μg), kanamycin (30 μg), lincomycin (2 μg), neomycin (30 μg), novobiocin (30 μg), oxacillin (1 μg), penicillin G (10 IU), streptomycin sulfate (10 μg), tetracycline (30 μg) and vancomycin (30 μg). +, Positive; W, weakly positive; S, slowly positive; SW, slowly and weakly positive; −, negative.

Characteristic	1	2	3	4	5	6	7
Relationship with O_2	Strictly aerobic	Strictly aerobic	Facultatively aerobic	Strictly aerobic	Facultatively aerobic	Facultatively aerobic	Facultatively aerobic
H_2S production (cysteine)	+	−	+	+	+	+	+
H_2S production (thiosulfate)	+	+	+	+	+	+	+
Nitrate reduction	+	−	+	−	+	−	+
ONPG	+	−	+	−	+	+	−
Oxidase	+	+	W	−	W	W	−
Methyl red test	−	W	+	S	+	+	+
Voges–Proskauer test	−	−	−	−	+	−	−
Hydrolysis of:							
Aesculin	S	+	+	−	+	−	−
Casein	−	−	−	+	−	−	−
DNA	+	W	W	−	+	W	W
Gelatin	−	−	−	+	−	−	+
Hippurate	+	−	+	+	+	+	+
Milk	−	−	−	+	−	−	SW
Pectin	−	−	−	+	−	+	+
Starch	−	W	−	+	W	+	+
Xylan	−	−	−	+	−	+	+
Utilization of (acid production from) single carbon source							
D-Arabinose	−(−)	−(−)	+(+)	+(+)	+(+)	−(−)	+(+)
Cellobiose	−(−)	+(W)	+(+)	+(+)	+(+)	−(−)	−(−)
D-Galactose	W(−)	−(−)	+(+)	+(+)	+(+)	+(+)	+(+)
Lactose	−(−)	−(−)	−(−)	−(−)	+(W)	−(−)	−(−)
D-Lyxose	+(+)	−(−)	+(−)	W(−)	+(+)	−(−)	+(−)
D-Mannose	+(−)	+(+)	+(+)	+(+)	+(+)	−(−)	+(+)
Melezitose	W(+)	−(−)	W(−)	W(−)	+(+)	−(−)	W(W)
Melibiose	W(+)	−(−)	−(−)	−(−)	+(W)	−(−)	−(−)
Raffinose	SW(−)	−(−)	−(−)	−(−)	+(+)	S(−)	W(−)
L-Rhamnose	−(−)	+(+)	−(−)	+(W)	−(−)	−(−)	−(−)
Ribose	−(−)	+(+)	−(−)	+(+)	W(W)	+(+)	+(+)

Table 1. cont.

Characteristic	1	2	3	4	5	6	7
D-Xylose	w(w)	−(−)	+(+)	+(+)	+(+)	−(−)	+(+)
Adonitol	−(−)	+(w)	−(−)	+(+)	−(−)	+(+)	+(+)
Glycerol	+(+)	+(w)	+(w)	+(+)	+(+)	−(−)	+(+)
Inositol	w(−)	+(w)	−(−)	+(+)	+(+)	−(−)	−(−)
Mannitol	+(w)	−(−)	+(w)	+(+)	−(−)	+(+)	−(−)
Sorbitol	−(−)	w(w)	−(−)	−(−)	−(−)	−(−)	−(−)
Xylitol	−(−)	+(w)	−(−)	−(−)	−(−)	−(−)	−(−)
Salicin	w(w)	w(w)	+(+)	−(−)	+(+)	−(−)	+(+)
Citrate	−	+	+	−	+	−	−
Fumarate	+	+	+	+	+	+	−
Gluconate	w	−	+	+	+	+	−
Malate	w	+	+	+	+	w	−
Propionate	w	+	w	+	+	−	w
Succinate	w	+	+	+	+	w	−
L-Arginine	−	−	+	+	−	+	+
L-Lysine	−	−	−	−	−	−	w
L-Phenylalanine	−	−	+	+	+	+	+
L-Proline	−	−	+	+	+	+	+
Utilization of nitrogen source							
Asparagine	+	+	+	+	+	+	−
Caseine	−	−	−	+	−	−	−
Gelatin	−	−	−	+	−	−	s
Glutamine	+	+	w	+	+	−	+
Antibiotic sensitivity							
Bacitracin (0.04 U)	+	w	+	−	+	+	+
Nalidixic acid (30 μg)	+	+	+	−	w	+	+
0.001 % Lysozyme resistance	−	−	−	−	−	−	s
DNA G+C content (mol%)							
T_m method	38.3±0.98	38.6±0.15	44.8±0.32	42.7±0.12	42.8±0.84	43.7±0.52	44.4±0.60
HPLC method	39.2±0.95	39.3±1.06	44.5±0.30	40.9±0.37	42.9±1.06	42.9±1.72	43.3±0.16
DNA–DNA relatedness (%) with strain 3nP4^T	100	9.7	40.2	20.6	15.7	20.9	19.5

After 40 h incubation, strain 3nP4^T formed whitish-yellow, translucent colonies with waxy surface, serrated edge and diameters of approximately 3 mm. Cells of strain 3nP4^T were motile, spore-forming rods with a Gram-stain-positive cell wall. Ellipsoidal endospores were located terminally and not swollen (Fig. S1 available in IJSEM Online). Strain 3nP4^T grew within the temperature and pH ranges of 38–66 °C and pH 6.0–9.3 with optimal growth at 57–60 °C and pH 7.0–7.5. Although higher growth rates were detected with 0–0.5 % (w/v) NaCl, strain 3nP4^T could tolerate up to 4 % (w/v) NaCl.

Strain 3nP4^T was strictly aerobic. No growth was observed in anaerobic conditions (by anaerobic respiration with NO_3^-, Fe^{3+}, $S_2O_3^{2-}$ or SO_4^{2-} as electron acceptors or by fermentation). Growth of strain 3nP4^T was obligately dependent upon a source of vitamins (vitamin solution or yeast extract). Growth was observed only with vitamin mixture (Balch *et al.*, 1979) or $\geqslant$0.01 % yeast extract added. Detailed results of all physiological and biochemical examinations are summarized in Table 1.

Major polar lipids of strain 3nP4^T were diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and one unknown phospholipid (PL1), which correlated well with all reference *Anoxybacillus* strains except for *Anoxybacillus tepidamans* DSM 16325^T (Fig. S2). Instead of PL1, phosphatidylmethylethanolamine could be distinctively detected in this strain. Moreover, several glycolipids or aminoglycolipids were found in strain 3nP4^T and all reference *Anoxybacillus* strains tested here, never previously reported in species of the genus *Anoxybacillus*. *Aeribacillus pallidus* DSM 3670^T had a completely different polar lipid pattern (for example PE was absent), which could clearly distinguish it from the genus *Anoxybacillus*.

Fatty acid profiles of strain 3nP4^T and the reference strains are given in Table S1. All *Anoxybacillus* strains, as well as strain 3nP4^T, exhibited similar fatty acid composition, in which iso-$C_{15:0}$ dominated. Distinctively, the predominant fatty acid of *Aeribacillus pallidus* DSM 3670^T was $C_{16:0}$, in agreement with the profile provided by Miñana-Galbis *et al.* (2010).

Menaquinone 7 was detected as the predominant respiratory quinone for strain 3nP4^T. This is common for several thermophilic bacilli groups such as the genera *Geobacillus*, *Anoxybacillus*, *Aeribacillus* and *Caldibacillus*.

A 16S rRNA gene sequence of 1511 nt was determined for strain 3nP4^T. Pairwise sequence alignment indicated that the novel isolate was related most closely to the genus *Anoxybacillus* and shared the highest sequence similarity of 97.08 % with *Anoxybacillus voinovskiensis* DSM 12111^T. Also, strain 3nP4^T was related closely to *Aeribacillus pallidus* DSM 3670^T and *Saccharococcus thermophilus* DSM 4749^T (96.72 and 96.26 % 16S rRNA gene sequence similarity, respectively). Phylogenetic analysis based on multiple sequence alignment showed that strain 3nP4^T clustered with the *Anoxybacillus* lineage in both the neighbour-joining (Fig. 1) and the maximum-likelihood (Fig. S3) trees, although the corresponding bootstrap values were both lower than 50 %.

The DNA G+C content of strain 3nP4^T was 39.2$\pm$0.95 mol% (HPLC). DNA–DNA relatedness values between the novel isolate and the reference strains are given in Table 1, and were all lower than 50 %.

The result of RAPD analysis showed diverse genotypic characterization among strain 3nP4^T and the reference strains (Fig. S4). Internal transcribed spacer fingerprints among all tested strains were also completely different (Fig. S4).

Strain 3nP4^T possessed the typical polar lipid profile and fatty acid pattern of the genus *Anoxybacillus*, in which DPG, PE and PG and iso-$C_{15:0}$ are the major components, respectively. Therefore, although phylogenetic analysis indicated that strain 3nP4^T grouped within the radiation of the genus *Anoxybacillus* with low bootstrap support, it is proposed to represent a novel species of the genus *Anoxybacillus*. The isolation of other related strains might in future improve this level of bootstrap support.

Several characteristics could be used to distinguish strain 3nP4^T from the reference *Anoxybacillus* strains. Reliance on vitamin mixture or yeast extract for growth was the most distinctive physiological characteristic of the novel isolate. Strain 3nP4^T was more similar to *Aeribacillus pallidus* DSM 3670^T than to the reference *Anoxybacillus* strains in some other physiological characteristics, such as inhibition by $Pb(CH_3COO)_2$ used in the H_2S production test and no assimilation of L-phenylalanine or L-proline.

Based on the results of this taxonomic study using a polyphasic approach, strain 3nP4^T is considered to represent a novel species of the genus *Anoxybacillus*, for which the name *Anoxybacillus vitaminiphilus* sp. nov. is proposed.

Description of *Anoxybacillus vitaminiphilus* sp. nov.

Anoxybacillus vitaminiphilus [vi.ta.mi.ni′phi.lus. N.L. n. *vitaminum* vitamin; N.L. masc. adj. *philus* (from Gr. masc. adj. philos) friend, loving; N.L. masc. adj. *vitaminiphilus* vitamin-loving, referring to the vitamin requirements].

Motile and spore-forming rods about 0.8–1.0 μm wide and variable in length (2.5–7.5 μm). Ellipsoidal endospores are terminally inserted and not swollen. Gram-stain-positive. Strictly aerobic. After incubation for 40 h, colonies are whitish-yellow and translucent with waxy surface, serrated edge and approximately 3 mm in diameter. Growth occurs at 38–66 °C (optimum 57–60 °C), at pH 6.0–9.3 (optimum pH 7.0–7.5) and with 0–4 % (w/v) NaCl (optimum 0–0.5 %). Obligately requires vitamin mixture or yeast extract for growth. Negative result in methyl red and Voges–Proskauer reaction tests, and for citrate utilization, indole production, phenylalanine deamination

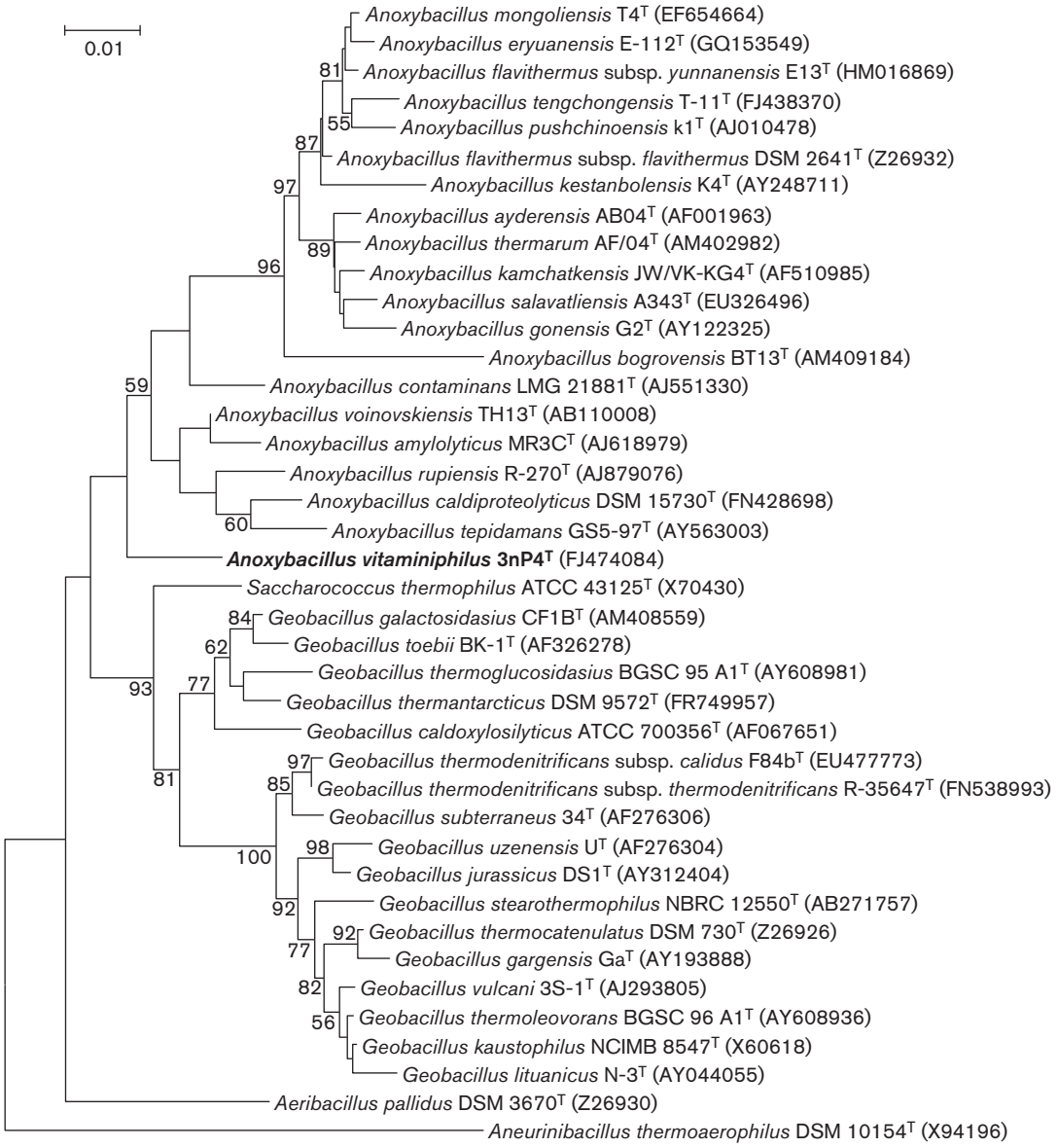

Fig. 1. Neighbour-joining phylogenetic tree showing the position of strain 3nP4^T among related taxa. Bootstrap values as percentages of 1000 replicates are given at branch points; only values >50 % are shown. Bar, 0.01 substitutions per nucleotide position.

and tyrosine degradation. Casein, gelatin, milk, pectin, starch, Tweens 20 and 80 and xylan are not hydrolysed. Positive result in tests for catalase, oxidase and ONPG, H_2S production and nitrate reduction. Positive for hydrolysis of aesculin (slowly), DNA, hippurate and Tweens 40 and 60. Utilizes D-fructose, D-galactose, D-glucose, D-lyxose, maltose, D-mannose, melezitose, melibiose, raffinose, sucrose, trehalose, D-xylose, glycerol, inositol, mannitol, salicin, acetate, fumarate, gluconate, malate, propionate, pyruvate, succinate, L-asparagine, L-aspartic acid, L-glutamate and L-glutamine, but not D-arabinose, cellobiose, lactose, L-rhamnose, ribose, L-sorbose, adonitol, dulcitol, erythritol, formate, sorbitol, xylitol, citrate, L-arginine, L-lysine, L-methionine, L-phenylalanine, L-proline or L-serine. Acid is produced from D-fructose, D-glucose, D-lyxose, maltose, melezitose, melibiose, sucrose, trehalose, D-xylose, glycerol, mannitol and salicin. Assimilates asparagine, glutamine, Casamino acids, peptone, tryptone, yeast extract, urea and ammonium sulfate as single nitrogen source. Sensitive to ampicillin (10 μg), bacitracin (0.04 U), carbenicillin (100 μg), chloramphenicol (30 μg), erythromycin (15 μg), gentamicin (10 μg), kanamycin (30 μg), lincomycin (2 μg), nalidixic acid (30 μg), neomycin (30 μg), novobiocin (30 μg), oxacillin (1 μg), penicillin G (10 IU), streptomycin sulfate (10 μg), tetracycline (30 μg) and vancomycin (30 μg). Major polar lipids are DPG, PG, PE and PL1. Minor polar lipids include

aminoglycolipid, aminophospholipid, glycolipid, aminolipid and PL2. The major fatty acids are iso-$C_{15:0}$ and iso-$C_{17:0}$.

The type strain, 3nP4^T (=CGMCC 1.8979^T=JCM 16594^T), was isolated from a water sample of the Puge hot spring, Sichuan province, south-west China. The DNA G+C content of the type strain is 39.2±0.95 mol% (HPLC).

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