# *Paenibacillus algorifonticola* sp. nov., isolated from a cold spring

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A Gram-stain-positive, endospore-forming, rod-shaped bacterium, designated XJ259$^T$, was isolated from a cold spring sample from Xinjiang Uyghur Autonomous Region, China. The isolate grew optimally at 20–30 °C and pH 7.3–7.8. Comparative analysis of the 16S rRNA gene sequence showed that isolate XJ259$^T$ belonged phylogenetically to the genus *Paenibacillus*, and was most closely related to *Paenibacillus xinjiangensis* B538$^T$ (with 96.6 % sequence similarity), *Paenibacillus glycanilyticus* DS-1$^T$ (96.3 %) and *Paenibacillus castaneae* Ch-32$^T$ (96.1 %), sharing less than 96.0 % sequence similarity with all other members of the genus *Paenibacillus*. Chemotaxonomic analysis revealing menaquinone-7 (MK-7) as the major isoprenoid quinone, diphosphatidylglycerol, phosphatidylethanolamine and two unknown phosphoglycolipids as the major cellular polar lipids, a DNA G+C content of 47.0 mol%, and anteiso-$C_{15:0}$ and $C_{16:0}$ as the major fatty acids supported affiliation of the new isolate to the genus *Paenibacillus*. Based on these data, isolate XJ259$^T$ is considered to represent a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus algorifonticola* sp. nov. is proposed. The type strain is XJ259$^T$ (=CGMCC 1.10223$^T$ =JCM 16598$^T$).

The genus *Paenibacillus* was delineated from members of group 3 *Bacillus* based on 16S rRNA gene sequence analysis by Ash *et al.* (1993), and the proposal was validly published in 1994. Following the establishment of the genus, more species were recognized and some characteristics in the original genus description were not found in several species. Therefore, the description of the genus *Paenibacillus* was emended by Shida *et al.* (1997). Furthermore, *Paenibacillus polymyxa* (Ash *et al.*, 1993, 1994) was considered as the type species of the genus by the Judicial Commission in 2005 (Judicial Commission of the International Committee for Systematics of Prokaryotes, 2005). At the time of writing, 117 species with validly published names were recognized as members of the genus *Paenibacillus* (http://www.bacterio.cict.fr/p/paenibacillus.html). Species belonging to this genus were mostly aerobic or facultatively anaerobic, rod-shaped, endospore-forming bacteria, possessing anteiso-$C_{15:0}$ as the major cellular fatty acid and having genomic DNA G+C contents in the range 39–54 mol% (Shida *et al.*, 1997; Montes *et al.*, 2004; Takeda *et al.*, 2005). They had been isolated from various habitats such as warm springs (Saha *et al.*, 2005; Chou *et al.*, 2007), food (Berge *et al.*, 2002), cow faeces (Velázquez *et al.*, 2004), Antarctic sediment (Montes *et al.*, 2004), phyllosphere (Rivas *et al.*, 2005, 2006; Valverde *et al.*, 2008), alkaline soil (Yoon *et al.*, 2005), Pu'er tea

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain XJ259$^T$ is GQ383922.

Three supplementary figures are available with the online version of this paper.

(Kim *et al.*, 2009), rhizosphere (Daane *et al.*, 2002; Kuisiene *et al.*, 2008), insects (Park *et al.*, 2009) and clinical specimens such as human blood (Ko *et al.*, 2008; Roux & Raoult, 2004) or human cerebrospinal fluid (Roux *et al.*, 2008). In this paper, the taxonomic position of a cold spring isolate, XJ259$^T$, which was found to be phylogenetically related to members of the genus *Paenibacillus*, was determined by using a polyphasic taxonomic approach and by comparisons based on preliminary 16S rRNA gene sequence analysis with reference strains belonging to the most closely related species of the genus *Paenibacillus*, *Paenibacillus xinjiangensis*, *Paenibacillus castaneae* and *Paenibacillus glycanilyticus*.

Strain XJ259$^T$ was isolated from a cold spring water sample from the city Urumchi of Xinjiang Uyghur Autonomous Region by dilution-plating on TYEG agar and incubating at 10 °C for 3 days. TYEG agar contained 10 g tryptone, 5 g yeast extract, 5 g glucose, 3 g $K_2HPO_4$ and 20 g agar in 1000 ml distilled water, pH 7.0. The isolate was routinely maintained on TYEG agar slants at 4 °C and preserved as suspensions of cells in glycerol (20 %, v/v) at −70 °C, as were the three closely related type strains, *P. xinjiangensis* B538$^T$ (=DSM 16970$^T$), *P. castaneae* Ch-32$^T$ (=DSM 19417$^T$) and *P. glycanilyticus* DS-1$^T$ (=NBRC 16618$^T$), which were supplied by the German Resource Centre for Biological Material (DSMZ) and the NITE Biological Resource Centre (NBRC).

The 16S rRNA gene sequence of strain XJ259$^T$ (1519 nt) was determined and analysed as described by Lane (1991), with some modifications. For initial taxonomic classification of the sequence, a combination of BLAST search (Altschul *et al.*, 1997, http://www.ncbi.nlm.nih.gov/blast) and the IDENTIFY program of the online server of EzTaxon (http://www.eztaxon.org/) was used (Chun *et al.*, 2007). For phylogenetic analysis, 16S rRNA gene sequences of type strains of all recognized species of the genus *Paenibacillus* downloaded from DDBJ/EMBL/GenBank were used; sequences shorter than 1300 nt or containing ambiguous nucleotides were excluded. A similarity matrix of all 16S rRNA gene sequences was generated after multiple alignment of the data by using CLUSTAL X (Thompson *et al.*, 1997), and, accordingly, 19 of the most closely related sequences were chosen to make a relatively small tree. The 16S rRNA gene sequences of *Oxalophagus oxalicus* DSM 5503$^T$ (Y14581), *Ammoniphilus oxalaticus* RAOx1$^T$ (Y14578) and *Aneurinibacillus aneurinilyticus* DSM 5562$^T$ (X94194) were selected as an outgroup. Three tree reconstruction methods were employed in this study. The neighbour-joining tree (Saitou & Nei, 1987) was calculated by using distances corrected according to the Kimura two-parameter model (Kimura, 1980, 1983) with the software package MEGA version 4.0 (Kumar *et al.*, 2008; Tamura *et al.*, 2007). For reconstruction of the maximum-parsimony tree, the software package PHYLIP version 3.6 (Felsenstein, 2002) was used. For reconstruction of the maximum-likelihood tree, the online version of PhyML (Guindon *et al.*, 2005) was used. The topology of the trees was evaluated by performing a bootstrap analysis (Felsenstein, 1985) of 1000 resamplings. The DNA G+C content of strain XJ259$^T$ was determined by using the thermal denaturation method (Mandel & Marmur, 1968), with *Escherichia coli* K-12 (CGMCC 1.748) as the reference, employing a PerkinElmer LAMBDA 25 UV/Vis spectrophotometer with a thermal controller.

A range of chemotaxonomic studies was carried out to confirm whether the chemical profile of strain XJ259$^T$ supported its assignment to the genus *Paenibacillus*. Cellular biomass for chemical studies was obtained by cultivation in shaken flasks (150 r.p.m.) with TYEG broth (pH 7.5) at 25 °C for 3 days unless indicated otherwise. Analysis of whole-cell fatty acids of isolate XJ259$^T$ and the three reference strains was

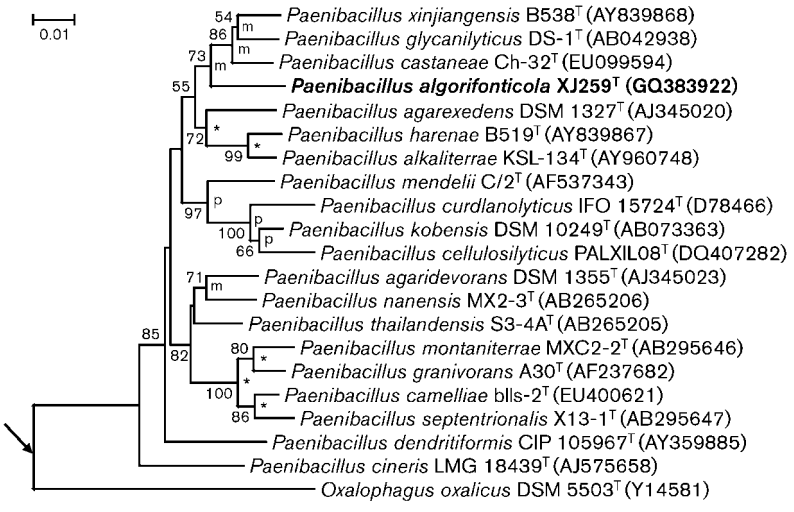


**Fig. 1.** Neighbour-joining tree showing phylogenetic relationships between strain XJ259$^T$ and the most closely related species belonging to the genus *Paenibacillus* based on 16S rRNA gene sequences. Asterisks at nodes indicate branches recovered with all three methods; m and p indicate branches which are also recovered by using the maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) treeing algorithms, respectively. Arrow indicates the estimated position of the three root organisms, including *O. oxalicus* DSM 5503$^T$ (Y14581), *Ammoniphilus oxalaticus* RAOx1$^T$ (Y14578) and *Aneurinibacillus aneurinilyticus* DSM 5562$^T$ (X94194). Bootstrap values are shown as percentages of 1000 replicates; only values >50 % are shown. Bar, 0.01 substitutions per nucleotide position.

performed according to the Microbial Identification System (MIDI; Microbial ID) after cultivation in TSB (tryptic soy broth; BD Bacto) for 2 days at 25 °C. Polar lipids were extracted and examined by one- and two-dimensional TLC on Merck silica gel 60 F254 aluminium-backed thin-layer plates according to the procedures described by Kates (1986) and Collins *et al.* (1980). Freeze-dried cells were collected and analysis of the peptidoglycan was carried out by using the method described by Schleifer & Kandler (1972), with the modification that TLC was performed on a microcrystalline cellulose plate. Isoprenoid quinones were determined as described by Komagata & Suzuki (1987).

Gram staining of strain XJ259$^T$ was performed according to the modified method of Hucker (1921). Cell morphology was observed by using a light microscope (Olympus CX41) and a transmission electron microscope (Hitachi H600) as described by Jeon *et al.* (2005). Motility was observed at 12 and 36 h with the light microscope. Anaerobic growth was tested as described by Valverde *et al.* (2008). Growth was tested at different temperatures (4–55 °C) and at different pH (4.0–11.0) at 25 °C in TYEG broth. Media with different pH were prepared by using appropriate biological buffers; $Na_2HPO_4/NaH_2PO_4$, $Na_2CO_3/NaHCO_3$ and $Na_2HPO_4$/NaOH were used for pH below 8.0, pH 8.0–10.0 and pH 11.0, respectively (Gomori, 1955). Requirements for and tolerance of NaCl were determined in TYEG medium supplemented with NaCl (0–10 %, w/v). Oxidase and catalase activities, hydrolysis of casein, gelatin and Tweens 20, 40 and 80, Methyl Red/Voges–Proskauer reaction, indole test, $H_2S$ production and nitrate reduction were determined as reported by Barrow & Feltham (1993). Hydrolysis of starch was tested by using starch agar with 95 % ethanol and Gram's iodine (Claus & Berkeley, 1986). Metabolism of citrate was tested by using Simmons' citrate medium (Smibert & Krieg, 1981). Acid production from L-rhamnose, D-mannose, D-ribose, D-galactose, D-arabinose, D-fructose, raffinose, D-xylose, L-sorbose, maltose, sucrose, lactose, D-glucose, inositol, trehalose, mannitol and glycerol was evaluated in basal medium (beef extract, 0.3 %; tryptose, 1 %; NaCl, 0.5 %) containing 0.003 % (w/v) bromophenol blue and 1 % each carbohydrate according to the modified method of Leifson (1963) after 3 days of cultivation at 25 °C. Utilization of 96 substrates as sole carbon source was tested using Biolog GP2 plates.

Comparisons with 16S rRNA gene sequences from the GenBank/EMBL/DDBJ database revealed that the novel strain (1519 nt) had the highest similarity to *P. xinjiangensis* B538$^T$ (96.6 %) (Lim *et al.*, 2006), followed by *P. glycanilyticus* DS-1$^T$ (96.3 %) (Dasman *et al.*, 2002) and *P. castaneae* Ch-32$^T$ (96.1 %) (Valverde *et al.*, 2008). 16S rRNA gene sequence similarities of strain XJ259$^T$ with the other recognized species of the genus *Paenibacillus* were less than 96.0 %. Neighbour-joining phylogenetic analysis based on 16S rRNA gene sequence analysis revealed that strain XJ259$^T$ formed a robust cluster with the three most closely related species of the genus *Paenibacillus* (Fig. 1 and Supplementary Fig. S1, available in IJSEM Online); the bootstrap value obtained was above 70 % in the small tree (Fig. 1). Phylogenetic trees built by using the maximum-parsimony and maximum-likelihood algorithms were similar to the neighbour-joining tree and also supported inclusion of isolate XJ259$^T$ in the genus *Paenibacillus*.

The DNA G+C content of isolate XJ259$^T$ was 47.0 mol%. Strain XJ259$^T$ contained anteiso-$C_{15:0}$ (63.2 %), $C_{16:0}$ (13.7 %), iso-$C_{16:0}$ (6.0 %), iso-$C_{15:0}$ (4.7 %) and iso-$C_{14:0}$ (4.6 %) as the major cellular fatty acids; other components were less than 4.0 %. This profile was consistent with those of closely related neighbours tested in parallel (Table 1), but, unlike the reference strains, the proportion of saturated straight-chain fatty acid $C_{16:0}$ was relatively high

**Table 1.** Cellular fatty acid profiles of strain XJ259$^T$ and related type strains

Strains: 1, *P. algorifonticola* sp. nov. XJ259$^T$; 2, *P. xinjiangensis* B538$^T$ (=DSM 16970$^T$); 3, *P. glycanilyticus* DS-1$^T$ (=NBRC 16618$^T$); 4, *P. castaneae* Ch-32$^T$ (=DSM 19417$^T$). Values shown are percentages of total fatty acids. –, Not detected. All strains were tested concurrently.

| Fatty acid | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| **Saturated straight-chain** | | | | |
| $C_{10:0}$ | – | 1.13 | – | 1.70 |
| $C_{12:0}$ | 0.14 | 1.30 | 0.24 | 0.58 |
| $C_{14:0}$ | 3.50 | 3.36 | 1.39 | 1.39 |
| $C_{15:0}$ | – | – | – | – |
| $C_{16:0}$ | **13.69** | **9.60** | **7.77** | **5.41** |
| $C_{17:0}$ | – | – | – | – |
| $C_{18:0}$ | – | – | – | 0.08 |
| **Saturated iso-branched** | | | | |
| iso-$C_{11:0}$ | 0.10 | – | – | – |
| iso-$C_{13:0}$ | – | 0.15 | – | 0.47 |
| iso-$C_{14:0}$ | 4.58 | 3.17 | 3.54 | 1.64 |
| iso-$C_{15:0}$ | 4.71 | **7.17** | 4.52 | **8.76** |
| iso-$C_{16:0}$ | **6.01** | 4.70 | **20.38** | 3.29 |
| iso-$C_{17:0}$ | 1.38 | 2.48 | 1.06 | 1.46 |
| iso-$C_{19:0}$ | – | – | 0.07 | – |
| **Saturated anteiso-branched** | | | | |
| anteiso-$C_{11:0}$ | 0.06 | 0.98 | 0.82 | 0.43 |
| anteiso-$C_{13:0}$ | 0.09 | 0.25 | – | 0.80 |
| anteiso-$C_{15:0}$ | **63.21** | **60.24** | **56.56** | **71.27** |
| anteiso-$C_{17:0}$ | 2.25 | 2.86 | 3.12 | 2.57 |
| **Unsaturated** | | | | |
| 11-Methyl $C_{18:1}\omega7c$ | 0.08 | – | – | – |
| $C_{16:1}\omega7c$ alcohol | – | 0.26 | – | – |
| $C_{16:1}\omega11c$ | – | 2.05 | – | – |
| $C_{20:1}\omega7c$ | – | – | 0.06 | – |
| **Hydroxy** | | | | |
| iso-$C_{11:0}$ 3-OH | 0.06 | 0.20 | – | 0.08 |
| iso-$C_{13:0}$ 3-OH | – | – | 0.35 | – |
| iso-$C_{14:0}$ 3-OH | – | 0.10 | – | – |
| $C_{15:0}$ 2-OH | – | – | – | 0.06 |
| **Summed feature*** | | | | |
| 3 | 0.07 | – | 0.12 | – |

*Summed feature 3 consists of $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$.

in strain XJ259$^T$. Isolate XJ259$^T$ contained MK-7 as the major isoprenoid quinone, in accordance with other representatives of the genus *Paenibacillus*. Diphosphatidylglycerol, phosphatidylethanolamine and two unknown phosphoglycolipids were detected as the major polar lipids. The diagnostic cell-wall diamino acid in the peptidoglycan layer of isolate XJ259$^T$ was *meso*-diaminopimelic acid, and the major whole-cell sugars were rhamnose and ribose.

Isolate XJ259$^T$ grew well on TSA (tryptic soy agar; BD Bacto), nutrient agar (NA; BD Bacto), LB agar and TYEG agar, and formed smooth, circular to fried-egg-formed and cream coloured colonies on all agars in this study after 3 days of incubation at 25 °C. The strain grew at 10–37 °C (optimum, 20–30 °C), pH 7.0–8.0 (optimum, pH 7.3–7.8) and 0–3 % (w/v) NaCl (optimum, 0–1 %). Isolate XJ259$^T$ was Gram-stain-positive, oxidase-negative, catalase-positive, and positive for hydrolysis of Tweens 20 and 40. Freshly isolated cells were rod-shaped (0.7–1.0 μm wide and 2.0–3.2 μm long) and motile with peritrichous flagella (Supplementary Fig. S2). Ellipsoidal endospores were produced after 4 days of growth on 10-fold diluted TYEG medium supplemented with 5 mg $MnSO_4$ $l^{-1}$ (Supplementary Fig. S3). Biolog GP2 plate results revealed that isolate XJ259$^T$ could use α-cyclodextrin, cyclodextrin, dextrin, arbutin, cellobiose, D-fructose, gentiobiose, α-D-glucose, maltose, maltotriose, D-mannose, melibiose, D-psicose and salicin as sole carbon source. Other phenotypic characters of strain XJ259$^T$ and comparisons with strains of related species are detailed in Table 2. All the phenotypic data of strain XJ259$^T$ were in accordance with its assignment to the genus *Paenibacillus*, and obvious differences were observed from the related strains (Table 2). Colonies of strain XJ259$^T$ grew firmly on all agars tested and were very hard to scrape off, whereas colonies of the other strains grew softly on agar plates. Anaerobic growth was observed for strain XJ259$^T$ but not for the other strains. Strain XJ259$^T$ was found to grow within a comparatively narrow pH range (7.0–8.0), whereas the other strains could grow at relatively wide ranging pH and appeared moderately acid-tolerant. Besides, the acid-production pattern of strain XJ259$^T$ from a range of carbon sources was different from those of other species of the genus *Paenibacillus*, e.g. acid was not produced from trehalose by strain XJ259$^T$, but was produced by the other strains tested.

All of the characteristics determined for strain XJ259$^T$ were in accordance with those for the genus *Paenibacillus*. However, the phylogenetic distances from recognized species of the genus *Paenibacillus* and the combination of unique phenotypic characteristics indicated that strain XJ259$^T$ is not affiliated to any of these recognized species. Therefore, it is concluded that strain XJ259$^T$ represents a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus algorifonticola* sp. nov. is proposed.

**Table 2.** Physiological properties of strain XJ259$^T$ and type strains of related species of the genus *Paenibacillus*

Strains: 1, *P. algorifonticola* sp. nov. XJ259$^T$; 2, *P. xinjiangensis* B538$^T$ (=DSM 16970$^T$); 3, *P. glycanilyticus* DS-1$^T$ (=NBRC 16618$^T$); 4, *P. castaneae* Ch-32$^T$ (=DSM 19417$^T$). +, Positive reaction; −, negative reaction. All strains were positive for catalase and hydrolysis of Tweens 20 and 40. All strains were negative for nitrate reduction, $H_2S$ production, and hydrolysis of citrate, casein and Tween 80. Acids were produced by all strains from the following carbon sources: D-glucose, D-mannose, D-galactose, D-arabinose, raffinose, D-xylose, maltose, sucrose and lactose. Acids were not produced by all strains from the following carbon sources: L-sorbose and inositol.

| Characteristic | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Colony colour | Cream | White–cream | Pinkish yellow | Yellow |
| Endospore position | Terminal | Terminal | Subterminal | Subterminal |
| Anaerobic growth | + | − | − | − |
| Oxidase | − | − | − | + |
| Hydrolysis of: | | | | |
| Starch | + | + | + | − |
| Gelatin | − | − | − | + |
| Acid production from: | | | | |
| D-Ribose | + | + | + | − |
| D-Fructose | + | + | + | − |
| Trehalose | − | + | + | + |
| Mannitol | + | − | + | + |
| Glycerol | − | + | + | − |
| Tolerance to NaCl (%, w/v) | 0–3 | 0–3 | 0–5 | 0–2.5 |
| Temperature range (°C) | 10–37 | 10–40 | 10–40 | 4–40 |
| Optimum growth temperature (°C) | 20–30 | 30–35 | 28–37 | 15–30 |
| pH range | 7.0–8.0 | 6.5–9.5 | 5.5–8.0 | 5.5–8.5 |
| DNA G+C content (mol%) | 47.0 | 47.0 | 50.5 | 46.0 |

## Description of *Paenibacillus algorifonticola* sp. nov.

*Paenibacillus algorifonticola* [al.go.ri.fon.ti′co.la. L. n. *algor* the cold; L. n. *fons fontis* a spring; L. suff. *-cola* (from L. masc. or fem. n. *incola*) an inhabitant of a place, a resident; N.L. n. *algorifonticola* an inhabitant of a cold spring].

Cells are rod-shaped, approximately 0.7–1.0 μm wide and 2.0–3.2 μm long, facultatively anaerobic, Gram-stain-positive, and motile with peritrichous flagella. Ellipsoidal spores are produced within a swollen sporangium and situated terminally. Catalase-positive and oxidase-negative. Colonies are smooth, circular to fried-egg-formed and cream coloured on TYEG agar. Growth occurs at 10–37 °C (optimum, 20–30 °C), with 0–3 % (w/v) NaCl (optimum, 0–1 %) and at pH 7.0–8.0 (optimum, pH 7.3–7.8). Hydrolysis of starch and Tweens 20 and 40 is positive. Hydrolysis of Tween 80, citrate, casein and gelatin is negative. Nitrate is not reduced to nitrite. Methyl Red/Voges–Proskauer reaction, indole test and $H_2S$ production are negative. Acids are produced from D-mannose, D-ribose, D-galactose, D-arabinose, D-fructose, raffinose, D-xylose, maltose, sucrose, lactose, D-glucose and mannitol, but not from L-sorbose, inositol, trehalose or glycerol. Cells can use α-cyclodextrin, cyclodextrin, dextrin, arbutin, cellobiose, D-fructose, gentiobiose, α-D-glucose, maltose, maltotriose, D-mannose, melibiose, D-psicose and salicin as sole carbon source. Cell wall contains *meso*-diaminopimelic acid and rhamnose. The predominant menaquinone is MK-7. The major cellular fatty acids are anteiso-$C_{15:0}$ and $C_{16:0}$. Diphosphatidylglycerol, phosphatidylethanolamine and two unknown phosphoglycolipids are the major polar lipids. The DNA G+C content of the type strain is 47.0 mol%.

The type strain is XJ259$^T$ (=CGMCC 1.10223$^T$ =JCM 16598$^T$), isolated from a cold spring from Xinjiang Uyghur Autonomous Region in China.

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