Thermomonas carbonis sp. nov., isolated from the soil of a coal mine

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Strain GZ436^T was Gram-stain-negative, aerobic, non-motile, rod-shaped and isolated from the soil of a coal mine. 16S rRNA gene phylogenetic analysis showed that this strain clustered with *Thermomonas brevis* LMG 21746^T (97.5 %), *Thermomonas haemolytica* A50-7-3^T (96.3 %), *Thermomonas koreensis* KCTC 12540^T (96.4 %), *Thermomonas hydrothermalis* SGM-6^T (95.5 %) and *Thermomonas fusca* LMG 21737^T (95.1 %). The major isoprenoid quinone was Q-8. The DNA G+C content was 67 mol%. Strain GZ436^T contained phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, an unknown aminophospholipid, an unknown phospholipid and an unknown lipid as the major polar lipids. The predominant cellular fatty acids (>5 %) were iso-$C_{15:0}$, iso-$C_{11:0}$, iso-$C_{11:0}$ 3-OH, iso-$C_{17:1}\omega9c$, $C_{16:0}$ and summed feature 3. The DNA–DNA relatedness value between strain GZ436^T and *T. brevis* LMG 21746^T was 54 ± 0.4 %. According to phenotypic and phylogenetic characteristics, strain GZ436^T represents a novel species of the genus *Thermomonas*, for which the name *Thermomonas carbonis* sp. nov. is proposed. The type strain is GZ436^T (=CCTCC AB 2013364^T=KCTC 42013^T).

The genus *Thermomonas*, belonging to the family *Xanthomonadaceae*, was firstly established and described by Busse *et al.* (2002). At the time of writing, this genus comprises five species isolated from a wide range of habitats, such as kaolin slurry [the type species, *Thermomonas haemolytica* (Busse *et al.*, 2002)], a hot spring [*Thermomonas hydrothermalis* (Alves *et al.*, 2003)]; a denitrification reactor [*Thermomonas brevis* and *Thermomonas fusca* (Mergaert *et al.*, 2003)] and a ginseng field [(*Thermomonas koreensis* (Kim *et al.*, 2006)]. The range of temperatures over which these species grow differs and they are either mesophilic or slightly thermophilic (Mergaert *et al.*, 2003). *T. haemolytica* and *T. hydrothermalis* have optimum temperatures for growth between 37 °C and 50 °C (Busse *et al.*, 2002; Alves *et al.*, 2003) However, the other three members of the genus *Thermomonas* have relatively low optimum growth temperatures of between 28 °C and 37 °C (Mergaert *et al.*, 2003; Kim *et al.*, 2006). Compared with the other members of the family *Xanthomonadaceae*, the main differences between members of the genus *Thermomonas* lie in their 16S rRNA gene phylogeny and their optimum growth temperatures. The characteristics, which are common to members of the genus *Thermomonas* are that they are Gram-stain-negative, rod-shaped, non-motile, aerobic, oxidase- and catalase-positive, have ubiquinone Q-8 as the major isoprenoid quinone, phosphatidylglycerol (PG), diphosphatidylglycerol (DPG) and phosphatidylethanolamine (PE) as the predominant polar lipids, and iso-branched fatty acids as the major fatty acids (Busse *et al.*, 2002; Alves *et al.*, 2003; Mergaert *et al.*, 2003; Kim *et al.*, 2006). The DNA G+C content of strains of species of the genus *Thermomonas* range from 64.7 to 68.7 mol% (Busse *et al.*, 2002; Alves *et al.*, 2003; Mergaert *et al.*, 2003; Kim *et al.*, 2006). Micro-organisms that are Gram-stain-negative, rod-shaped, aerobic and catalase-positive, with ubiquinone Q-8 and iso-branched fatty acids are also common within the family *Xanthomonadaceae* (Kumari *et al.*, 2011; Zhang *et al.*, 2011).

While investigating the micro-organisms of a soil sample from Baiping coal mine (27° 15′ 00″ N 106° 24′ 06″ E), Bijie city, Guizhou province, PR China, a strain designated GZ436^T was isolated. Isolation was conducted at 28 °C using the dilution plating method with 1/10 TSA medium (tryptic soy broth agar, pH 7.0; Difco, BD).

The 16S rRNA gene sequence was amplified, as described by Fan *et al.* (2008), and compared with the 16S rRNA sequences supplied in the EzTaxon-e server (Kim *et al.*, 2012). Multi-alignments with closely related strains were carried out using the CLUSTAL X program (Thompson *et al.*, 1997). Phylogenetic trees were then reconstructed using neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods with MEGA4.0 (Tamura

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Abbreviations: DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain GZ436^T is KF923805.

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

et al., 2007), and the maximum-likelihood (Felsenstein, 1981) algorithm with the PHYML online web server (Guindon *et al.*, 2010).

The maximum-likelihood phylogenetic tree, using the almost-complete 16S rRNA gene sequence (1468 bp) of strain GZ436^T, showed that the strain was grouped with five members of the genus *Thermomonas* (Fig. 1). The five type strains were *T. brevis* LMG 21746^T (97.5 % sequence similarity), *T. haemolytica* A50-7-3^T (96.4 %), *T. koreensis* KCTC 12540^T (96 %), *T. hydrothermalis* SGM-6^T (95.5 %) and *T. fusca* LMG 21737^T (95.1 %). Strain GZ436^T also showed high similarities with *Lysobacter antibioticus* DSM 2044^T (96.4 %), *Lysobacter capsici* YC5194^T (96.3 %) and *Lysobacter gummosus* KCTC 12132^T (96.2 %), but they were located within different clusters (Fig. 1). The neighbour-joining tree (Fig. S1; available with the online Supplementary Material) and the maximum-parsimony tree (Fig. S2) both supported the phylogenetic position obtained with the maximum-likelihood method (Fig. 1). Multi-alignments of 16S rRNA gene sequences showed that strain GZ436^T contained 16 major conserved nucleotides, which were the same in the five strains representing species of the genus *Thermomonas*, but differed from the strains representing species of the genus *Lysobacter* (Fig. S3). Therefore, these five type strains of species of the genus *Thermomonas* were used as reference strains in the following studies.

The morphological, physiological and biochemical characteristics of strain GZ436^T and the five reference strains were analysed together. Cell morphology was examined using light microscopy and transmission electron microscopy H-7650 (Hitach, Japan) with cells cultivated for 3 days on R2A (l^{-1}: 0.5 g yeast extract, 0.5 g proteose-peptone, 0.5 g Casamino acids, 0.5 g glucose, 0.5 g soluble

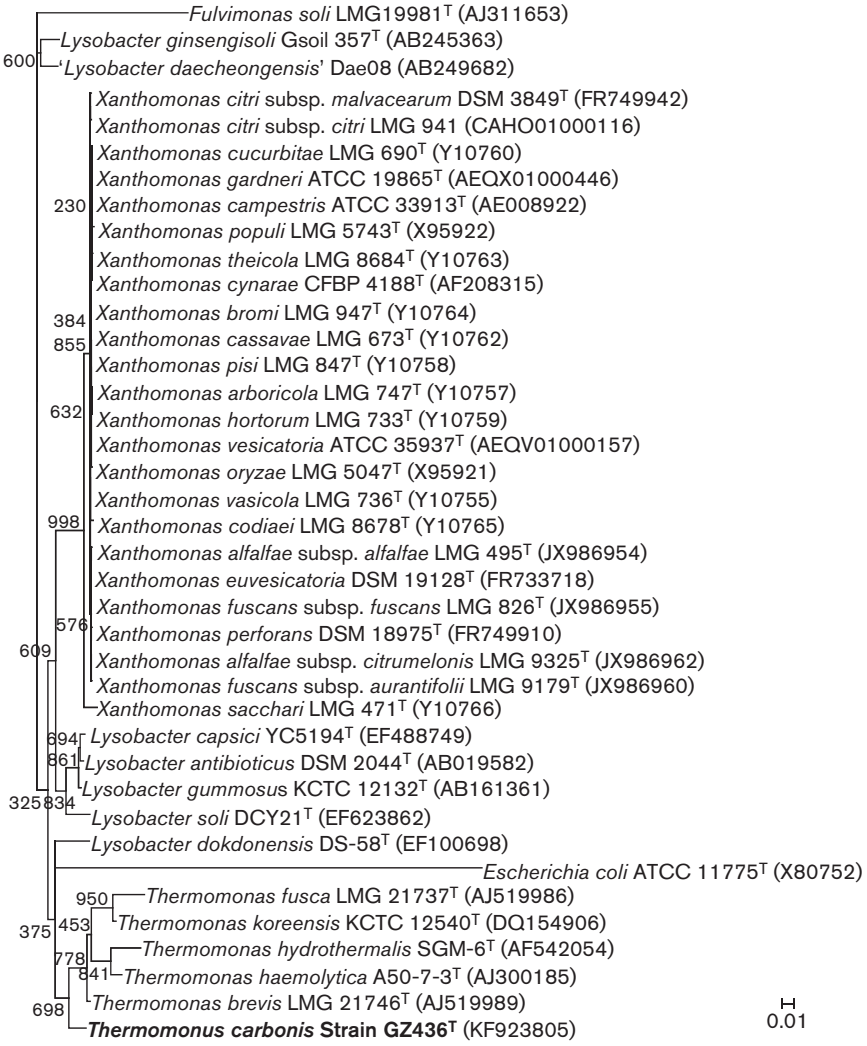

Fig. 1. A maximum-likelihood phylogenetic tree reconstructed on the basis of 16S rRNA gene sequences. Numbers at the nodes indicate bootstrap values expressed as percentages of 1000 replications. Bar, 1 substitution per 100 nt.

starch, 0.3 g sodium pyruvate, 0.3 g K_2HPO_4, 0.05 g $MgSO_4$, pH 7.0–7.2) (Difco) at 28 °C. Motility was tested on R2A medium with 0.3 % agar. Gram staining was tested as described by Dussault (1955) combined with the KOH lysis method (Ryu, 1938). Growth at various temperatures (4, 16, 28, 32, 35, 37, 40 and 50 °C) and NaCl concentrations (0–5 %, w/v) was assessed after 7 days of incubation on R2A. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber on R2A and on R2A supplemented with nitrate. Growth was also tested on nutrient agar (NA), full-strength trypticase soy agar (TSA), Luria-Bertani (LB) agar and MacConkey agar (all Difco). R2A at pH 4–10 (at intervals of one pH unit) was prepared with the following buffer systems: pH 4.0–7.0, 0.1 M citric acid/0.2 M Na_2HPO_4; pH 8.0–9.0, 0.2 M Tris/0.2 M HCl; pH 10.0, 0.05 M $NaHCO_3$/0.1 M NaOH. Production of H_2S was assessed according to the method of Dong & Cai (2001). Haemolysis was conducted on a Columbia agar base (Oxoid) supplemented with 10 % (v/v) defibrinated sheeps' blood after 3 days of incubation at 28 °C. Catalase activity was determined by assessing bubble production from 3 % (v/v) H_2O_2 and oxidase activity was assessed using 1 % (w/v) tetramethyl-β-phenylenediamine (Merck). Acid production from various carbohydrates was tested as described by Hugh & Leifson (1953). Other physiological and biochemical characteristics and enzyme activities were determined using the API 20 NE, API ID 32 GN and API ZYM systems according to the manufacturer's instructions (bioMérieux). Sole nitrogen and carbon source utilization was determined with 0.1 % (w/v) of each of the sources (urea, potassium nitrate, glutamate, ammonium nitrate, ammonium, sodium nitrite, histidine, L-proline, L-serine, L-threonine and cysteine), as described by Dong & Cai (2001).

Cells of strain GZ436^T were ovoid rod-shaped, 0.7–0.9 μm × 0.3–0.4 μm, and non-motile. Colonies grown on R2A plates for three days were smooth, circular and yellow. Cells were Gram-stain-negative. Growth occurred between 4 and 32 °C and at pH 5–9, and was optimal at 28 °C and pH 7. No growth occurred under anaerobic conditions on R2A or on R2A supplemented with nitrate. Detailed

Table 1. Differential phenotypic characteristics of strain GZ436^T and related species of the genus *Thermomonas*

Strains: 1, GZ436^T; 2, *T. brevis* DSM 15422^T; 3, *T. haemolytica* DSM 13605^T; 4, *T. koreensis* KCTC 12540^T; 5, *T. hydrothermalis* DSM 14834^T; 6, *T. fusca* DSM 15424^T. All taxa are Gram-stain-negative rods and are catalase- and oxidase-positive. +, Positive; −, negative; W, weakly positive. All data are from this study.

Characteristic	1	2	3	4	5	6
Yellow pigment	+	−	−	−	−	−
Colony colour on R2A	Yellow	Beige	Cream	Beige	Cream	Beige
Brown pigment on R2A at 28 °C or 37 °C	−	−	−	+	−	W
Nitrate reduction	−	−*	−	+	+	+
Nitrite reduction	+	+	−	−	+	+
H_2S production	−	−	+	+	−	+
Optimum growth temperature (°C)	28	28–37	37–50	37	50	28–37
Haemolysis	−	−	+	+	+	−
Aesculin hydrolysis	+	+	−	+	+	−
Assimilation tests (API 20NE and 32GN)						
D-Glucose	−	+	−	+	+	−
D-Maltose	W	+	−	+	+	−
D-Sucrose	+	−	−	−	−	−
D-Mannitol	+	−	−	−	−	−
D-Sorbitol	+	−	−	−	−	−
L-Xylose	+	−	−	−	−	−
N-Acetylglucosamine	+	+	−	−	−	−
D-Mannose	−	+	−	−	−	−
L-Threonine	−	−	−	+	−	+
L-Proline	+	+	−	+	+	+
L-Phenylalanine	+	+	+	+	−	+
Enzyme activities (API ZYM)						
N-Acetylglucosamine	+	+	−	−	−	−
Trypsin	W	+	−	−	−	−
β-Glucosidase	−	−	−	−*	+	−
Cystine arylamidase	W	+	W	+	−	+
Chymotrypsin	W	−	W	−	W	+*

*Data different from studies of Busse *et al.* (2002), Mergaert *et al.* (2003), Alves *et al.* (2003) and Kim *et al.* (2006).

morphological, physiological and biochemical characteristics of strain GZ436^T are given in the species description. Strain GZ436^T could be distinguished from the five type strains of species of the genus *Thermomonas* in utilizing D-mannitol, L-xylose and D-sorbitol as sole carbon resources (Table 1).

DNA–DNA hybridization experiments were performed with *T. brevis* LMG 21746^T using the thermal denaturation and renaturation method (Huss *et al.*, 1983) and the DNA G+C content was determined by HPLC, as described by Mesbah *et al.* (1989). The respiratory quinones were extracted and identified by HPLC as described by Minnikin *et al.* (1984). For cellular fatty acids analysis, strain GZ436^T and the reference strains *T. brevis* LMG 21746^T, *T. haemolytica* A50-7-3^T, *T. koreensis* KCTC 12540^T, *T. hydrothermalis* SGM-6^T and *T. fusca* LMG 21737^T were analysed by GC (model 6890; Hewlett Packard) according to the instructions of the Sherlock Microbial Identification System [Sherlock version 4.5 (0209B), TSBA40 4.10; MIDI] (Sasser, 1990). Polar lipids analysis was determined by two-dimensional TLC as described by Collins & Jones (1980).

The DNA–DNA relatedness between strain GZ436^T and *T. brevis* LMG 21746^T was 54.4 %±0.4 (n=2). The DNA G+C content of strain GZ436^T was 67 mol%. Similarly to the type species *T. haemolytica* and the other members of the genus *Thermomonas*, strain GZ436^T possessed Q-8 as the major respiratory quinone. The predominant cellular fatty acids of strain GZ436^T were iso-$C_{15:0}$, iso-$C_{11:0}$, iso-$C_{11:0}$ 3-OH, iso-$C_{17:1}\omega9c$, $C_{16:0}$ and summed feature 3 (Table 2). Compared with the five type strains of the genus *Thermomonas*, strain GZ436^T contained higher amounts of summed feature 3 (17.5 %), which comprises $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH. The major polar lipids found in strain GZ436^T were PG, DPG, PE, an unknown aminophospholipid (APL), an unknown phospholipid (PL) and an unknown lipid (L), while in the most closely related reference strain, *T. brevis* LMG 21746^T, they were PG, DPG, PE, APL, an unknown amino lipid (AL) and L. (Fig. S4).

On the basis of the close relationship and the distinctive phenotypic and phylogenetic characteristics of strain GZ436^T and other species of the genus *Thermomonas*, it is concluded that strain GZ436^T represents a novel species of the genus *Thermomonas*, for which the namehttp://dx.doi.org/10.1601/nm.2261 *Thermomonas carbonis* sp. nov. is proposed.

Description of *Thermomonas carbonis* sp. nov.

Thermomonas carbonis (car.bo′nis. L. gen. n. *carbonis* of coal, referring to the isolation site of the type strain).

Cells are Gram-stain-negative, aerobic, non-motile and rod-shaped (0.7–0.9 μm × 0.3-0.4 μm). Colonies are yellow, convex, circular, smooth and non-transparent after incubation on R2A at 28 °C for 3 days. Growth occurs on full-strength TSA, nutrient agar, R2A agar and LB agar but not on MacConkey agar. The temperature range for growth is 4–32 °C (optimal at 28 °C) and the pH range for growth is pH 6–9 (optimum pH 7.0). NaCl is not obligatory for growth. Shows protease (gelatin hydrolysis) activity and does not show arginine dihydrolase, urease or β-galactosidase (PNPG) activity. Positive for catalase, oxidase, C4-esterase, alkaline phosphatase, C8-esterase, leucine, arylamidase, valine, arylamidase, naphthol-AS-BI-phosphohydrolase and *N*-acetylglucosaminidase, weakly positive for acid phosphatase, trypsin, cystine arylamidase, chymotrypsin and α-glucosidase, and negative for lipase (C14), β-glucuronidase, α-galactosidase, β-glucosidase, α-mannosidase and β-fucosidase activities. Negative reaction for glucose fermentation, indole production, H_2S production and nitrate reduction. Does not hydrolyse CM-cellulose, starch, DNase, Tween 80 or Tween 20. Assimilates D-maltose, acetate, citrate, D-sucrose, L-xylose, D-sorbitol, D-mannitol, caprate, *myo*-inositol, 2-ketogluconate, 3-hydroxybutyrate, 3-hydroxybenzoate, L-proline, L-histidine, L-alanine, L-serine and L-phenylalanine. Does not assimilate D-glucose, L-arabinose, D-mannose, gluconate, adipate, malate, phenylacetate, L-rhamnose, D-ribose, itaconate, suberate, malonate, lactate, 5-ketogluconate, glycogen, L-threonine, D-melibiose, L-fucose or valerate. The polar lipids are PG, DPG, PE, an unknown aminophospholipid, an unknown phospholipid and an unknown lipid. The major cellular fatty acids (>5 %) are iso-$C_{15:0}$, iso-$C_{11:0}$, iso-$C_{11:0}$ 3-OH, iso-$C_{17:1}\omega9c$, $C_{16:0}$ and summed feature 3. The predominant respiratory quinone is Q-8.

Table 2. Cellular fatty acid profiles of strain GZ436^T and related species of the genus *Thermomonas*

Strains: 1, GZ436^T; 2, *T. brevis* DSM 15422^T; 3, *T. haemolytica* DSM 13606^T; 4, *T. koreensis* KCTC 12540^T; 5, *T. hydrothermalis* DSM 14834^T; 6, *T. fusca* DSM 15424^T. Values represent percentages of total fatty acids. All data are from this study. −, <1 % Or not detected.

Fatty acid	1	2	3	4	5	6
Saturated fatty acids						
iso-$C_{11:0}$	8.8	11.0	9.7	8.7	9.5	11.4
iso-$C_{13:0}$	−	−	−	2.9	−	5.0
$C_{14:0}$	3.2	1.3	−	1.7	−	2.0
iso-$C_{14:0}$	1.4	1.7	2.9	2.6	−	4.4
iso-$C_{15:0}$	35.8	44.3	48.7	47.4	52.3	38.2
anteiso-$C_{15:0}$	−	1.1	−	−	−	−
$C_{16:0}$	6.0	1.9	1.4	−	2.0	−
iso-$C_{16:0}$	2.7	3.0	10.4	1.5	1.2	1.8
iso-$C_{17:0}$	1.2	1.7	3.9	−	7.6	−
Unsaturated fatty acids						
iso-$C_{15:1}$ F*	3.1	1.8	1.0	8.3	1.2	5.5
iso-$C_{17:1}\omega9c$	7.9	17.6	8.0	16.1	13.8	16.5
Hydroxy fatty acids						
iso-$C_{11:0}$3-OH	7.8	8.6	10.6	5.9	8.0	8.8
Summed feature 3†	17.5	2.2	−	1.5	−	2.3

*iso-$C_{15:1}\omega6c$ and/or iso-$C_{15:1}\omega5c$ (Yassin *et al.*, 2007).
†Represents two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 3 comprises $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH.

The type strain is GZ436^T (=CCTCC AB 2013364^T= KCTC 42031^T), which was isolated from the soil of a coal mine in Bijie city, Guizhou province, PR China. The DNA G+C content of the type strain is 67 mol%.

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