Thermomonas koreensis sp. nov., a mesophilic bacterium isolated from a ginseng field

Myung Kyum Kim,¹ Wan-Taek Im,¹ Jun-Gyo In,² Sung-Hoon Kim³ and Deok-Chun Yang¹

Correspondence

Deok-Chun Yang
deokchunyang@yahoo.co.kr

^{1,3}Department of Oriental Medicinal Material & Processing, College of Life Science¹, and Department of Oncology, Graduate School of East-West Medical Science³, Kyung Hee University, 1 Seocheon, Kiheung Yongin, Kyunggi 449-701, South Korea

²Biopia Co., Ltd, 383-4 Singal-ri, Kiheung Yongin, Kyunggi-do 449-598, South Korea

A Gram-negative, non-spore-forming, rod-shaped, motile bacterium, strain Ko06^T, was isolated from soil from a ginseng field in South Korea and was characterized in order to determine its taxonomic position. 16S rRNA gene sequence analysis revealed that strain Ko06^T belongs to the *Gammaproteobacteria*, and the highest levels of sequence similarity were with *Thermomonas brevis* LMG 21746^T (98.4%), *Thermomonas fusca* LMG 21737^T (97.7%), *Thermomonas haemolytica* A50-7-3^T (96.5%) and *Thermomonas hydrothermalis* SGM-6^T (95.8%). Chemotaxonomic data revealed that strain Ko06^T possesses ubiquinone Q-8 and that the predominant fatty acids are C_{15:0} iso, C_{11:0} iso and C_{11:0} iso 3-OH, all of which corroborated assignment of the strain to the genus *Thermomonas*. The results of DNA–DNA hybridization and physiological and biochemical tests clearly demonstrated that strain Ko06^T represents a distinct species. On the basis of these data, strain Ko06^T (=KCTC 12540^T=NBRC 101155^T) should be classified as the type strain of a novel *Thermomonas* species, for which the name *Thermomonas koreensis* sp. nov. is proposed.

The genus *Thermomonas* was established by Busse *et al.* (2002) for four strains with high G + C contents isolated from kaolin slurry, in which the strains were growing at temperatures as high as 50 °C; *Thermomonas haemolytica* is the type species. At the time of writing, there are four *Thermomonas* species with validly published names: *Thermomonas haemolytica* (Busse *et al.*, 2002), *Thermomonas brevis* (Mergaert *et al.*, 2003), *Thermomonas fusca* (Mergaert *et al.*, 2003) and *Thermomonas hydrothermalis* (Alves *et al.*, 2003).

In a series of studies, we used a culture-dependent method in attempts to isolate micro-organisms from a ginseng field in order to investigate the make-up of the microbial community. In this study, one strain was isolated from soil from a ginseng field in Daejeon (South Korea) and characterized by using polyphasic approaches. These polyphasic approaches, including phylogenetic analysis based on 16S rRNA gene sequences, analysis of genomic relatedness and assessment of chemotaxonomic and phenotypic properties, were conducted to determine the precise taxonomic position of strain Ko06^T. The results obtained in this study indicated that Ko06^T can be assigned as a member of the

genus *Thermomonas* but is clearly distinguishable from the *Thermomonas* species with validly published names.

Strain Ko06^T was isolated, using direct plating onto R2A agar (Difco), from soil from a ginseng field near Daechung Lake. Single colonies on these plates were purified by transferring them onto new plates and subjecting them to an additional incubation for 3 days at 30 °C. The purified colonies were tentatively identified by means of partial 16S rRNA gene sequences.

Cell morphology and motility were observed with a Nikon light microscope (1000 × magnification), with the cells being allowed to grow for 3 days at 30 °C on R2A agar. Gram reactions were conducted according to the non-staining method, as described by Buck (1982). Oxidase activity was evaluated using the oxidation of 1% *p*-aminodimethylaniline oxalate. Catalase activity was determined by measuring bubble production after the application of a 3% (v/v) hydrogen peroxide solution. Acid production from carbohydrates was assessed by using the procedures outlined by Cappuccino & Sherman (2002). Growth at various temperatures (4, 15, 25, 30, 37, 42 and 50 °C) was assessed on R2A agar, and growth at various pH values was assessed in R2A broth. Growth on nutrient agar, trypticase soy agar and MacConkey agar (Difco) was also evaluated at 30 °C. The API 20NE and API ID 32GN systems were employed

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Ko06^T is DQ154906.

in these tests, according to the recommendations of the manufacturer (bioMérieux).

Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), purified using TLC and subsequently analysed by HPLC, as described previously (Collins & Jones, 1981; Shin *et al.*, 1996). For fatty acid methyl ester analyses, strain Ko06^T was allowed to grow on trypticase soy agar for 48 h at 30 °C and then two loops of the well-grown cells were harvested. Fatty acid methyl esters were prepared, separated and identified with the Sherlock Microbial Identification System (MIDI) (Sasser, 1990). Total cellular lipids were extracted and analysed using TLC (Kim *et al.*, 2005).

Genomic DNA of strain Ko06^T was extracted and purified with the Qiagen Genomic-tip system 100/G and then enzymically degraded into nucleosides, as described previously (Tamaoka & Komagata, 1984; Mesbah *et al.*, 1989). DNA–DNA hybridization was performed fluorometrically, according to the method developed by Ezaki *et al.* (1989), using photobiotin-labelled DNA probes and microdilution wells. Five replicate hybridizations were performed for each sample. The highest and lowest values obtained for each sample were excluded and the means of the remaining three values are quoted as DNA–DNA relatedness values.

Genomic DNA was extracted and purified with a genomic DNA isolation kit (Core Bio System). The 16S rRNA gene

was amplified from the chromosomal DNA of strain Ko06^T, using the universal bacterial primer set 9F and 1512R (Weisburg *et al.*, 1991); the purified PCR products were sequenced by Genotec (Daejeon, South Korea) (Kim *et al.*, 2005). The full sequence of the 16S rRNA gene was compiled with SeqMan software, and the 16S rRNA gene sequence of the test strain was edited using the BioEdit program (Hall, 1999). The 16S rRNA gene sequences of related taxa were obtained from GenBank. Multiple alignments were performed with the CLUSTAL X program (Thompson *et al.*, 1997). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). The phylogenetic tree was constructed using the neighbour-joining method (Saitou & Nei, 1987) in the MEGA 2 program (Kumar *et al.*, 2001). Bootstrap analysis with 1000 replicates was also conducted to obtain confidence levels for the branches (Felsenstein, 1985). All of the species in the genus *Thermomonas* were included in the phylogenetic tree.

Strain Ko06^T was cultured on R2A agar at 30 °C, yielding light-brownish and circular colonies. Strain Ko06^T was found to be an aerobic, Gram-negative, motile, rod-shaped bacterium. It was also determined as being able to grow at 20–37 °C, but it did not grow at 4 or 50 °C. The physiological characteristics of strain Ko06^T are summarized in the species description, and a comparison of distinguishing

Table 1. Differential phenotypic characteristics for strain Ko06^T and related *Thermomonas* species

Taxa: 1, *T. koreensis* sp. nov. Ko06^T; 2, *T. fusca* ($n=18$; Mergaert *et al.*, 2003); 3, *T. brevis* ($n=4$; Mergaert *et al.*, 2003); 4, *T. haemolytica* ($n=4$; Busse *et al.*, 2002); 5, *T. hydrothermalis* ($n=2$; Alves *et al.*, 2003). All taxa are Gram-negative rods and are catalase- and oxidase-positive. Symbols: +, all positive; –, all negative; (+), more than 60% of strains positive; (–), more than 60% of strains negative; w, weakly positive; ND, not determined.

Characteristic	1	2	3	4	5
Motility	+	+	+	+	–
Filamentous	–	+	–	+	–
Cell size (µm)	0.7 × 3–4	0.7 × 3–10	0.7 × 3–10	0.5–0.8 × 1.0–12.5	0.6–0.9 × 2.0–4.0
Brown pigment on R2A at 14–20 °C	w	+	–	ND	w
Optimum growth temperature (°C)	37	28–37	28–37	37–50	50
Growth temperature range (°C)	20–37	4–37	4–37	18–50	30–60
Nitrate reduction	+	+	+	–	+
Nitrite reduction	–	+	+	–	+
Enzyme activities					
β-Glucosidase	+	–	–	–	+
Lipase (C14)	–	(–)	–	+	+
Protease (gelatin hydrolysis)	+	+	+	(+)	+
Urease	–	–	–	+	–
Assimilation tests					
L-Malate	–	–	–	(–)	–
D-Glucose	+	–	+	–	+
D-Maltose	+	–	+	–	+
D-Mannose	–	–	+	–	–
N-Acetylglucosamine	–	–	+	–	–
DNA G + C content (mol%)	68.3	67.6–68.7	68.4	67.1–68.7	64.7

characteristics with those of related type strains is shown in Table 1.

The cellular fatty acid profiles of strain Ko06^T and related *Thermomonas* type strains are shown in Table 2. The major cellular fatty acid in strain Ko06^T was iso-pentadecanoic acid (C_{15:0} iso, 53.2%). Minor amounts of other iso-branched fatty acids, C_{16:0} 10-methyl (7.0%), C_{11:0} iso 3-OH (6.7%), C_{14:0} (5.3%), C_{15:0} anteiso (4.8%) and C_{11:0} iso (4.6%) were present. C_{11:0} iso 3-OH (6.7%) was the only hydroxy fatty acid found in strain Ko06^T. The presence of iso-branched fatty acids, with C_{15:0} iso as the major

fatty acid, is characteristic of genera in the *Xanthomonas* branch, including *Xanthomonas*, *Pseudoxanthomonas*, *Stenotrophomonas*, *Xylella* and *Luteimonas* (Assih *et al.*, 2002; Roumagnac *et al.*, 2004; Yang *et al.*, 2005). Significant differences in fatty acid profiles were found between different species of the genus *Thermomonas*.

The predominant quinone of strain Ko06^T was Q-8. The quinone system supported our assignment of strain Ko06^T to the *Xanthomonas* branch, in which the majority of species (including *Thermomonas* species) also exhibit Q-8 as the predominant quinone.

Table 2. Cellular fatty acid profiles of strain Ko06^T and related *Thermomonas* species

Taxa: 1, *T. koreensis* sp. nov. Ko06^T; 2, *T. fusca* (n=18; Mergaert *et al.*, 2003); 3, *T. brevis* (n=4; Mergaert *et al.*, 2003); 4, *T. haemolytica* (n=3; Mergaert *et al.*, 2003); 5, *T. hydrothermalis* (n=2; Alves *et al.*, 2003). Values are percentages of total fatty acids. For unsaturated fatty acids, the position of the double bond is taken from the terminal methyl group of the carbon chain; *cis* isomers are indicated by the suffix *c*. tr, Trace.

Fatty acid	1	2	3	4	5
Saturated fatty acids					
10:0 iso					0.5
11:0 anteiso					0.2–0.3
11:0 iso	4.6	5.8–9.1	7.2–10.1	7.9–8.4	4.8–5.0
12:0 iso					0.4
13:0 iso	3.2	0.6–4.6	tr	tr	0.6–1.0
14:0	5.3	1.4–2.8	1.1–1.3	tr	1.4–1.7
14:0 iso		0.9–8.1	tr	2.9–5.2	4.0–5.3
15:0					0.7–0.8
15:0 anteiso	4.8	0.3–2.2	tr	tr	3.7–4.0
15:0 iso	53.2	30.9–44.8	45.3–48.7	49.5–55.2	37.7–41.9
16:0	3.7	0.7–2.3	2.6–3.0	tr	2.3–3.3
16:0 10-methyl	7.0				
16:0 iso	3.9	0.6–11.2	tr	4.3–7.6	11.4–15.1
17:0 anteiso					0.2–0.6
17:0 iso		0.8–2.3	2.2–2.8	1.5–2.1	4.1–4.7
Unsaturated fatty acids					
14:1 ω 5 <i>c</i>					0.6–0.8
15:1 iso F	4.3	2.5–5.9	1.4–1.6	3.0–3.7	2.5–3.3
15:1 ω 6 <i>c</i>					0.8–0.9
16:1 iso H					0.5
16:1 ω 9 <i>c</i>					0.5–0.6
17:1 iso ω 9 <i>c</i>		14.4–23.3	20.1–22.5	11.3–12.3	0–5.3
Hydroxy fatty acids					
11:0 iso 3-OH	6.7	6.5–10.5	7.8–11.2	9.2–10.0	6.3–6.5
12:0 iso 3-OH					0.4
16:0 3-OH					0–0.3
Summed features*					
SF2					0.6–0.7
SF4	3.3	2.7–7.0	2.8–3.1	tr	4.6–4.8
SF5					0.1–0.2

*Summed features contain fatty acids that could not be separated by GLC with the Microbial Identification System (MIDI). SF2, 15:1 iso H and/or 13:0 3-OH; SF4, 16:1 ω 7*c* and/or 15:0 iso 2-OH; SF5, 17:1 iso I and/or 17:1 anteiso B.

Fig. 1. Phylogenetic tree, based on 16S rRNA gene sequences, showing the phylogenetic relationships between strain Ko06^T, all species of *Thermomonas* and members of related genera in the *Xanthomonas* branch. The neighbour-joining method was used. Bootstrap values higher than 66% are shown. Bar, 0.01 substitutions per nucleotide position.

The 16S rRNA gene sequence of strain Ko06^T was found to be a continuous stretch of 1474 nt. The 16S rRNA gene sequences of related taxa were obtained from GenBank. Strain Ko06^T was determined as belonging to the *Gamma-proteobacteria*, and the highest levels of sequence similarity were with *T. brevis* LMG 21746^T (98.4%), *T. fusca* LMG 21737^T (97.7%), *T. haemolytica* A50-7-3^T (96.5%) and *T. hydrothermalis* SGM-6^T (95.8%). In the phylogenetic tree (Fig. 1), strain Ko06^T clearly belongs to the *Thermomonas* lineage, as evident from the high bootstrap value.

The G + C content of the genomic DNA of strain Ko06^T was found to be 68.3 mol%. Strain Ko06^T exhibited a relatively low level of DNA–DNA relatedness to the type strains *T. fusca* LMG 21737^T (27%), *T. brevis* LMG 21746^T (13%), *T. haemolytica* A50-7-3^T (16%) and *T. hydrothermalis* SGM-6^T (19%). The level of DNA–DNA hybridization to related type strains was therefore less than 70% (Stackebrandt & Goebel, 1994), a value which is regarded as the threshold for delineating a genomic species. Our results therefore support the classification of strain Ko06^T within a separate, previously unrecognized species. The results of our polyphasic analysis support the recognition of a novel species within the genus *Thermomonas*, for which the name *Thermomonas koreensis* sp. nov. is proposed.

Description of *Thermomonas koreensis* sp. nov.

Thermomonas koreensis sp. nov. (ko.re.en'sis. N.L. fem. adj. *koreensis* pertaining to Korea, the location of the soil sample from which the type strain was isolated).

Cells are Gram-negative, aerobic, motile rods, 3.0–4.0 × 0.7 µm in size after growth on R2A agar for 3 days at 30 °C. The optimal growth temperature and pH are 37 °C and pH 8.0–9.0, and grow is observed at a salt concentration of 3%. Catalase- and oxidase-positive. Shows β-glucosidase and protease (gelatin hydrolysis) activity and does not show arginine dihydrolase, urease or β-galactosidase (PNPG) activity. Does not produce any biopolymer-hydrolysing enzymes such as cellulase, chitinase, DNase,

lipase or xylanase. Assimilates D-glucose, D-maltose, acetate, L-alanine, propionate, 3-hydroxybutyrate, L-proline and does not assimilate L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, gluconate, caprate, adipate, malate, citrate, phenylacetate, L-rhamnose, D-ribose, myo-inositol, D-sucrose, itaconate, suberate, malonate, lactate, 5-ketoglucuronate, glycogen, 3-hydroxybenzoate, L-serine, salicin, D-melibiose, L-fucose, D-sorbitol, valerate, L-histidine, 2-ketoglucuronate or 4-hydroxybenzoate. DNA G + C content is 68.3 mol% (as determined by HPLC). Q-8 is the predominant quinone. The major cellular fatty acid is C_{15:0} iso, (53.2%). Other phenotypic characteristics, such as substrates utilized and enzymes produced, are summarized in Table 1.

The type strain, Ko06^T (= KCTC 12540^T = NBRC 101155^T), was isolated from soil from a ginseng field near Daechung Lake in Daejeon, South Korea.

Acknowledgements

This work was supported by a grant (code MB-14) from the BioGreen 21 Program of the Rural Development Administration, Republic of Korea.

References

- Alves, M. P., Rainey, F. A., Nobre, M. F. & da Costa, M. S. (2003). *Thermomonas hydrothermalis* sp. nov., a new slightly thermophilic γ-proteobacterium isolated from a hot spring in central Portugal. *Syst Appl Microbiol* **26**, 70–75.
- Assih, E. A., Ouattara, A. S., Thierry, S., Cayol, J.-L., Labat, M. & Macarie, H. (2002). *Stenotrophomonas acidaminiphila* sp. nov., a strictly aerobic bacterium isolated from an upflow anaerobic sludge blanket (UASB) reactor. *Int J Syst Evol Microbiol* **52**, 559–568.
- Buck, J. D. (1982). Nonstaining (KOH) method for determination of Gram reactions of marine bacteria. *Appl Environ Microbiol* **44**, 992–993.
- Busse, H.-J., Kämpfer, P., Moore, E. R. B. & 7 other authors (2002). *Thermomonas haemolytica* gen. nov., sp. nov., a γ-proteobacterium from kaolin slurry. *Int J Syst Evol Microbiol* **52**, 473–483.

- Cappuccino, J. G. & Sherman, N. (2002).** *Microbiology: a Laboratory Manual*, 6th edn. Menlo Park, CA: Benjamin Cummings Science Publishing.
- Collins, M. D. & Jones, D. (1981).** Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implications. *Microbiol Rev* **45**, 316–354.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989).** Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Felsenstein, J. (1985).** Confidence limit on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Hall, T. A. (1999).** BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.
- Kim, M. K., Im, W.-T., Ohta, H., Lee, M. & Lee, S.-T. (2005).** *Sphingopyxis granuli* sp. nov., a β -glucosidase-producing bacterium in the family *Sphingomonadaceae* in α -4 subclass of the *Proteobacteria*. *J Microbiol* **43**, 152–157.
- Kimura, M. (1983).** *The Neutral Theory of Molecular Evolution*. Cambridge: Cambridge University Press.
- Kumar, S., Tamura, K., Jakobsen, I.-B. & Nei, M. (2001).** MEGA2: molecular evolutionary genetics analysis software. *Bioinformatics* **17**, 1244–1245.
- Mergaert, J., Cnockaert, M. C. & Swings, J. (2003).** *Thermomonas fusca* sp. nov. and *Thermomonas brevis* sp. nov., two mesophilic species isolated from a denitrification reactor with poly(ϵ -caprolactone) plastic granules as fixed bed, and emended description of the genus *Thermomonas*. *Int J Syst Evol Microbiol* **53**, 1961–1966.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989).** Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Roumagnac, P., Gagnevin, L., Gardan, L., Sutra, L., Manceau, C., Dickstein, E. R., Jones, J. B., Rott, P. & Pruvost, O. (2004).** Polyphasic characterization of xanthomonads isolated from onion, garlic and Welsh onion (*Allium* spp.) and their relatedness to different *Xanthomonas* species. *Int J Syst Evol Microbiol* **54**, 15–24.
- Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sasser, M. (1990).** *Identification of bacteria by gas chromatography of cellular fatty acids*. Technical Note 101. Newark, DE: MIDI.
- Shin, Y. K., Lee, J.-S., Chun, C. O., Kim, H.-J. & Park, Y.-H. (1996).** Isoprenoid quinone profiles of the *Leclercia adecarboxylata* KCTC 1036^T. *J Microbiol Biotechnol* **6**, 68–69.
- Stackebrandt, E. & Goebel, B. M. (1994).** Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Tamaoka, J. & Komagata, K. (1984).** Determination of DNA base composition by reversed phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997).** The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Weisburg, W. G., Barns, S. M., Pelletier, D. A. & Lane, D. J. (1991).** 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* **173**, 697–703.
- Yang, D.-C., Im, W.-T., Kim, M. K. & Lee, S.-T. (2005).** *Pseudoxanthomonas koreensis* sp. nov. and *Pseudoxanthomonas daejeonensis* sp. nov. *Int J Syst Evol Microbiol* **55**, 787–791.