Thalassomonas ganghwensis sp. nov., isolated from tidal flat sediment

Hana Yi,¹ Kyung Sook Bae² and Jongsik Chun¹

Correspondence
Jongsik Chun
jchun@snu.ac.kr

¹School of Biological Sciences, Seoul National University, 56-1 Shillim-dong, Kwanak-gu, Seoul 151-742, Korea

²Korean Collection for Type Cultures, Korea Research Institute of Bioscience and Biotechnology, Yusong, PO Box 115, Taejeon 305-600, Korea

A Gram-negative, aerobic, halophilic bacterium, designated strain JC2041^T, was isolated from a sediment sample of getbol, the Korean tidal flat. Results of 16S rDNA sequence analyses indicated a moderate relationship to *Thalassomonas viridans* within the γ -*Proteobacteria* (94.9% similarity). Depending on the tree-making algorithm used, the isolate either formed a monophyletic clade with *T. viridans* or was recovered as a sister group of a class containing the genera *Thalassomonas* and *Colwellia*. Phenotypic features of the getbol isolate were similar to those of *T. viridans*, but several physiological and chemotaxonomic properties, including nitrate reduction, amylase, lecithinase, Tweenase and utilization of 13 carbon sources, distinguished strain JC2041^T from *T. viridans*. The polyphasic data presented in this study indicate that the isolate should be classified as a novel species in the genus *Thalassomonas*. The name *Thalassomonas ganghwensis* sp. nov. is therefore proposed for the getbol isolate; the type strain is JC2041^T (=IMSNU 14005^T=KCTC 12041^T=DSM 15355^T).

In our recent study on the culturable aerobic bacterial community in getbol (Korean tidal flat), a large number of novel bacterial strains was isolated from Ganghwa Island in Korea (Yi & Chun, 2002). Five of these isolates were members of the γ -*Proteobacteria*, including strain JC2041^T and two strains of *Zooshikella ganghwensis* (Yi *et al.*, 2003). In this study, the polyphasic characterization of isolate JC2041^T is presented; the name *Thalassomonas ganghwensis* sp. nov. is proposed for this isolate.

Strain JC2041^T was isolated from a tidal flat sediment sample (37°36'22.3"N; 126°22'59.4"E) using MR2A [R2A (Difco) supplemented with the artificial sea water of Lyman & Fleming (1940)] at 25 °C and routinely maintained on MA (marine agar 2216; Difco) at 30 °C. *Thalassomonas viridans* DSM 13754^T was used as a reference strain and grown on MA at 30 °C.

Bacterial DNA preparation and PCR amplification and sequencing of 16S rDNA were carried out as described previously (Chun & Goodfellow, 1995). The resultant sequence of strain JC2041^T was aligned manually against sequences obtained from GenBank. Phylogenetic trees were inferred using the Fitch–Margoliash (Fitch & Margoliash,

1967), maximum-likelihood (Felsenstein, 1993), maximum-parsimony (Fitch, 1972) and neighbour-joining (Saitou & Nei, 1987) methods. Evolutionary distance matrices were generated according to Jukes & Cantor (1969). The resultant tree topologies were evaluated in bootstrap analyses (Felsenstein, 1985) of the neighbour-joining method based on 1000 resamplings. The alignment and phylogenetic analyses were carried out using PHYDIT (available at <http://plaza.snu.ac.kr/~jchun/phydit/>) and PAUP 4.0 (Swofford, 1998) as described previously (Chun *et al.*, 2000).

A nearly complete 16S rDNA sequence of strain JC2041^T was obtained (1408 bp) and used for an initial BLAST search against GenBank. The result clearly indicated that the getbol isolate was a member of the γ -*Proteobacteria*. The newly determined sequence was then aligned manually against representatives of the γ -*Proteobacteria* based on the secondary structure of bacterial 16S rRNA. Strain JC2041^T showed highest 16S rDNA sequence similarity to *T. viridans* CECT 5083^T (94.9%); the next highest similarities were observed with the genera *Colwellia* (93.0–93.4%), *Idiomarina* (90.9–91.1%) and *Pseudoalteromonas* (88.4–90.2%).

These relationships were also observed in phylogenetic trees, as shown in Fig. 1. The clade containing strain JC2041^T, *T. viridans* and *Colwellia* species had 100% bootstrap support and was obtained by all tree-making methods. However, the isolate either formed a monophyletic clade with *T. viridans* (in Fitch–Margoliash and

Fig. 1. Neighbour-joining tree based on nearly complete 16S rDNA sequences showing relationships among strain JC2041^T and members of the γ -Proteobacteria. Numbers at nodes are levels of bootstrap support (%) based on neighbour-joining analyses of 1000 resampled datasets; solid circles indicate that the corresponding nodes (groupings) are also recovered in Fitch–Margoliash, maximum-likelihood and maximum-parsimony trees. *Helicobacter pylori* ATCC 43504^T (U01330) was used as an outgroup (not shown). Bar, 0.1 nt substitutions per position.

neighbour-joining trees) or was recovered as a sister group to a clade containing *T. viridans* and *Colwellia* species (in maximum-parsimony and maximum-likelihood trees), depending on the tree-making algorithm used.

Cultural characteristics were studied using several bacteriological growth media. Although strain JC2041^T was isolated on MR2A, it grew better on MA, CSY-3 (Sawabe *et al.*, 1998) and SMM (Shioi's marine medium; Shiba, 1992). The test strain retained viability for about 20 days on MA at 30 °C. The isolate was strictly halophilic, requiring media containing 1–8 % (w/v) artificial sea salts (Sigma) for growth (optimum of 2–4 % sea salts). It was unable to grow on modified CSY-3 medium containing 0–15 % (w/v) NaCl alone. Growth on MA was observed in the pH range 7–11 and optimal growth occurred at pH 7–8. The temperature range for growth was 15–40 °C; optimum growth was observed at 35 °C. No growth was detected under anaerobic conditions created by a GasPack system (BBL).

The morphology of cells grown on MA at 30 °C was examined using phase-contrast microscopy, SEM and TEM. After 1 day incubation, colonies were approximately 2 mm in diameter; they reached a maximum diameter of 4–5 mm after 4 days. Colonies were circular, convex with an entire margin, glistening, translucent, viscid and yellowish. When grown on YBM [BM (basal medium; Baumann *et al.*, 1972) containing 0.01 % yeast extract] supplemented with 0.1 % tyrosine, strain JC2041^T produced diffusible brown pigments on an agar plate. Cells in late exponential phase were rod-shaped with a polar flagellum and approximately 1.5–2.3 μ m long by 0.5–0.8 μ m wide. Spore formation was not observed.

Carbon source utilization was tested on BM supplemented with 0.1 % yeast extract. Other physiological properties were tested using standard procedures, as described previously (Yi *et al.*, 2003). These reactions, as well as some other properties of strain JC2041^T, are indicated in the species description. The test strain could be differentiated from *T. viridans* and phylogenetically related genera by a number of characteristics (Table 1).

Chemotaxonomic characteristics were determined for cells grown at 30 °C for 2 days on MA or in MB (marine broth 2216). Analysis of fatty acid methyl esters was performed by GLC according to the Microbial Identification (MIDI) system. Saturated and monounsaturated fatty acids, namely 16:0 (22.3 %) and 18:1 ω 7c (11.3 %), were the most abundant, together with a mixture of 16:1 ω 7c and i-15:0 2-OH (20.6 %; the MIDI system could not differentiate between these two fatty acids). Smaller, albeit substantial, amounts of 10:0 (4.9 %), i-16:0 (7.1 %), 16:1 ω 9c (4.7 %) and 17:1 ω 8c (4.4 %) were also detected. This fatty acid composition was most similar to that observed in the genus *Thalassomonas* and clearly differentiated the isolate from other phylogenetically related genera (Table 1). Only minor quantitative differences in amounts of 15:0, 16:0, 17:1 ω 8c and 18:1 ω 7c were found between our isolate and *T. viridans*.

Isoprenoid quinones of the test strain and *T. viridans* DSM 13754^T were isolated from freeze-dried biomass according to Minnikin *et al.* (1984), purified on preparative TLC (silica gel F₂₅₄; Merck) and analysed by HPLC (Waters) equipped with a reverse-phase column (Spherisorb ODS-2 80; Waters) as described by Collins (1985). Ubiquinone-8 (Q-8) was the predominant isoprenoid quinone in both strains. The genus *Colwellia* is also reported to contain Q-8 as its major quinone (Yumoto *et al.*, 1998).

The DNA G+C content was determined by HPLC of deoxyribonucleosides as described by Mesbah *et al.* (1989) using a reverse-phase column (Supelcosil LC-18-S; Supelco). The G+C content of strain JC2041^T was 42 mol%.

Although the phylogenetic position of strain JC2041^T is somewhat different depending on the tree building method used for analysis, the close relationship of our isolate to *T. viridans* was obvious in sequence similarity comparisons and phylogenetic trees based on 16S rDNA. Moreover, our isolate showed phenotypic traits that were similar to those of *T. viridans*, rather than to *Colwellia* species. However, the low sequence similarity, 94.9 %, and many physiological and chemotaxonomic properties, i.e. nitrate reduction, amylase, lecithinase, Tweenase and utilization of 13 carbon sources, distinguished our isolate from *T. viridans* (Table 1). Based on the polyphasic evidence presented in this study, it is fair to conclude that strain JC2041^T merits novel species status in the genus *Thalassomonas*. The name *Thalassomonas ganghwensis* sp. nov. is proposed for strain JC2041^T.

Table 1. Characteristics that differentiate strain JC2041^T from *T. viridans* and phylogenetically related genera

Strain/taxa: 1, strain JC2041^T; 2, *Thalassomonas viridans*; 3, genus *Colwellia*; 4, genus *Idiomarina*. Data were taken from this and earlier studies (Bowman *et al.*, 1998; Deming *et al.*, 1988; Ivanova *et al.*, 2000; Macián *et al.*, 2001; Yumoto *et al.*, 1998). +, Positive reaction; –, negative reaction; w, weakly positive; v, variable; v+, more than half of the species are positive and the type species is positive; v–, fewer than half of the species are negative and the type species is negative; ND, no data available; PUFA, polyunsaturated fatty acids.

Characteristic	1	2	3	4
Pigment	Yellow	Green	Red, white	Yellow
Sea salts requirement	+	+	v	–
Oxygen requirement	Strict aerobe	Strict aerobe	Facultative anaerobe	Strict aerobe
Growth at:				
4 °C	–	–	+	+
37 °C	+	+	–	–
Nitrate reduction	+	–	+	ND
Carbohydrate fermentation	–	–	v+	–
Amylase	–	+	v+	–
β-Galactosidase	+	+	–	ND
Chitinase	–	–	v+	v
DNase	+	+	v–	+
Lecithinase	–	+	ND	ND
Tweenase	+	–	v+	+
Utilization of:				
Acetate	+	–	+	v
D-Cellobiose	–	+	–	ND
D-Fructose	–	w	v–	–
D-Galactose	+	–	–	ND
D-Glucose	+	w	v	–
D-Ribose	–	+	ND	ND
Glycine	w	–	ND	ND
L-Arginine	–	+	v	ND
L-Asparagine	w	–	v	ND
L-Lysine	w	–	ND	–
L-Tyrosine	+	–	ND	+
Succinate	w	–	+	+
Tartrate	w	–	v	ND
DNA G+C content (mol%)	42	48	35–42	48–50
Fatty acids (% of total):				
i-15:0	–	–	–	33.7–40.6
15:0	1.2	6.0–11.1	0.9–14.3	–
i-16:0	7.1	<1.0	0.0–10.3	–
16:1 ω 9 c	4.7	–	0.0–20.0	<1.0
16:1 ω 7 <i>i</i> -15:0 2-OH	20.6	21.2–28.4	6.0–56.8	7.0–8.3
16:0	22.3	11.2–13.7	13.5–33.2	4.6–6.3
i-17:0	–	–	–	11.9–12.5
17:1 ω 8 c	4.4	14.1–19.7	0.0–5.6	0.8–1.1
18:1 ω 7 c	11.3	3.2–6.1	0.0–4.2	5.9–6.7
PUFA	–	–	0.8–9.5	–

Description of *Thalassomonas ganghwensis* sp. nov.

Thalassomonas ganghwensis (gang.hwen'sis. N.L. fem. adj. *ganghwensis* named after Ganghwa Island in Korea, the geographical origin of the type strain).

Gram-negative. Oxidase- and catalase-positive. Strictly aerobic, chemoheterotrophic and halophilic. Cells are rod-shaped (1.5–2.3 × 0.5–0.8 µm) and motile with a polar flagellum. Colonies are circular, convex, glistening, translucent, viscid, yellowish and have an entire margin on

MA. Does not grow without sea water or the addition of artificial sea salts (1–8 %) to the medium. Grows at 15–40 °C and pH 7–11. Optimal growth is observed at 35 °C, pH 7–8 and 2–4 % artificial sea salts. Abundant growth is observed on MA, CSY-3 and SMM media. Spores are not formed. Reduces nitrate to nitrite. Negative for fermentation of glucose. Decomposes casein, DNA, aesculin, gelatin and Tween 80, but not agar, alginate, cellulose, chitin, lecithin or starch. Produces β -galactosidase, but not arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, acetoin, fluorescein, H₂S, indole or polyhydroxybutyrate. Produces alkaline phosphatase, esterase (C4), leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase, but not esterase lipase (C8), lipase (C14), cystine arylamidase, trypsin, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase or α -fucosidase. Utilizes acetate, D-galactose, D-glucose and L-tyrosine as sole carbon sources. Glycine, L-asparagine, L-lysine, succinate and tartrate are weakly utilized. Does not utilize acetamide, benzoate, citrate, D-cellobiose, D-fructose, D-mannitol, D-mannose, D-raffinose, D-ribose, D-salicin, D-sorbitol, D-trehalose, D-xylose, ethanol, inositol, inulin, 2-propanol, lactose, L-arginine, L-ascorbate, L-ornithine, L-rhamnose, polyethylene glycol, salicylate or thiamin. The major isoprenoid quinone is ubiquinone-8. The major fatty acids are 16:0, 18:1 ω 7c and a mixture of 16:1 ω 7c and i-15:0 2-OH.

The type strain, JC2041^T (=IMSNU 14005^T=KCTC 12041^T=DSM 15355^T), was isolated from sediment of getbol, the Korean tidal flat. The DNA G+C content of the type strain is 42 mol%.

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