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NOTE

Gramella jeungdoensis sp. nov., Isolated from a Solar Saltern in Korea

Yochan Joung[1], Haneul Kim[1], Taeyong Jang[1], Tae-Seok Ahn[2], and Kiseong Joh[1]*

[1]*Department of Bioscience and Biotechnology, Hankuk University of Foreign Studies, Gyeonggi 449-791, Republic of Korea*
[2]*Departement of Environmental Science, Kangwon National University, Chuncheon 200-701, Republic of Korea*

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A non-motile, Gram- stain-negative, yellow pigmented, rod-shaped bacterium, strain HMD3159^T, was isolated from a solar saltern in Korea. The major fatty acids were iso-$C_{15:0}$ (26.3%), iso-$C_{17:0}$ 3OH (12.1%), iso-$C_{16:0}$ (12.0%), summed feature 3 (comprising $C_{16:1}$ ω7*c* and/or $C_{16:1}$ ω6*c*; 11.0%) and summed feature 9 (iso-C17:1 ω9c and/or 10-methyl $C_{16:0}$; 10.0%). The major respiratory quinone was MK-6. The DNA G+C content was 40.9 mol%. The phylogenetic tree based on 16S rRNA gene sequences showed that strain HMD3159^T formed a lineage within the genus Gramella and closely related to *Gramella gaetbulicola* (95.5% sequence similarity), Gramella portivictoriae (94.9%), Gramella echinicola (94.6%), and Gramella marina (93.6%). On the basis of the evidence presented in this study, strain HMD3159^T represents a novel species of the genus Gramella, for which the name Gramella *jeungdoensis* sp. nov., is proposed. The type strain is HMD3159^T (=KCTC 32123^T =CECT 7683^T).

Keywords: *Gramella* sp. nov., 16S rRNA similarity, saltern

The genus *Gramella*, belonging to the family *Flavobacteriace*, was originally described by Nedashkovskaya *et al.* (2005). Members of the genus *Gramella* were characterized by yellow pigment, Na^+ ion requirement for growth and no flexirubin-type pigments. The genus *Gramella* consisted of 4 species: *Gramella echinicola* (Nedashkovskaya *et al.*, 2005), *G. marina* (Nedashkovskaya *et al.*, 2010), *G. portivictoriae* (Lau *et al.*, 2005), and *G. geatbuicola* (Cho *et al*., 2010). The members of the genus *Gramella* was isolated from the sea urchin (*G. echinicola* and *G. marina*) and marine sediment (*G. portivictoriae* and *G. geatbuicola*).

In the course of a study on the microbial diversity of a solar saltern from Jeungdo, Republic of Korea, a yellow-pigmented colony was isolated. The isolated strain HMD3159^T was incubated on marine agar (MA, Difco, USA) for 3 days at 25℃. The isolate was routinely cultured on MA at 25°C and the culture was suspended in glycerol suspension (20%, w/v) for storage at -80°C.

Almost-complete sequence of the 16S rRNA gene was obtained for strain HMD3159^T, as described previously (Cho and Giovannoni, 2003). Identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarity were performed by using the EzTaxon server (Chun *et al.*, 2007). Phylogenetic and molecular evolutionary analyses were conducted by using MEGA version 4 (Tamura *et al.*, 2007). Sequence comparisons showed that strain HMD3159^T was closely related to members of the genus *Gramella*. Phylogenetic trees were inferred using the maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Fitch, 1971) and neighbour-joining (Saitou and Nei, 1987) algorithms. The robustness of the topologies for the maximum-likelihood and neighbour-joining trees was evaluated by means of bootstrap analysis (Felsenstein, 1985) based on 1,000 resamplings of the sequences, respectively. All of the phylogenetic trees generated in this study (Fig. 1) indicated that a solar saltern strain belonged to the genus *Gramella*. Strain HMD3159^T formed a coherent clade with *G. gaetbulicola* (95.5%), *G. portivictoriae* (94.9%), and *G. marina* (93.6%) within the phylogenetically well-resolved *Gramella* clade. This phylogenetic inference, coupled with 16S rRNA gene sequence similarities of <97% (Wayne *et al.*, 1987) between strain HMD3159^T and the other *Gramella* species, suggested that the strain should be assigned to the genus *Gramella* as a representative of a novel species.

Cell morphology was examined by light microscopy. Gram staining was determined using the bioMérieux Gram Stain kit according to the manufacturer's instructions. Anaerobic growth was tested on MA at 25°C by using a GasPak EZ Anaerobic container System (BD) according to the manufacturer's instructions. Cellular pigments were extracted with acetone/methanol (1:1, v/v) and their absorption spectra were determined using a UV/visible spectrophotometer (UV 6101A; Shimadzu). The presence of flexirubin-type pigments was investigated by using the pathochromatic shift test with a KOH solution (Bernardet *et al.*, 2005). Catalase and oxidase tests were performed according to the standard methods (Macfaddin and Macfaddin, 1980). The temperature range and optimum for growth were assessed on MA at 4℃, 10-30℃ (at 5℃ intervals), 37°C, 42°C, 45°C, and 50°C. The pH range for growth was determined in 1/10 dilution marine broth (Difco), adjusted to pH 4.0-11.0 (at intervals of 1.0 pH unit). Growth in the presence addition of 0.5% and 1.0-16.0% (at intervals of

* For correspondence. E-mail: ksjoh@hufs.ac.kr; Tel.: +82-31-330-4271; Fax: +82-31-330-4566

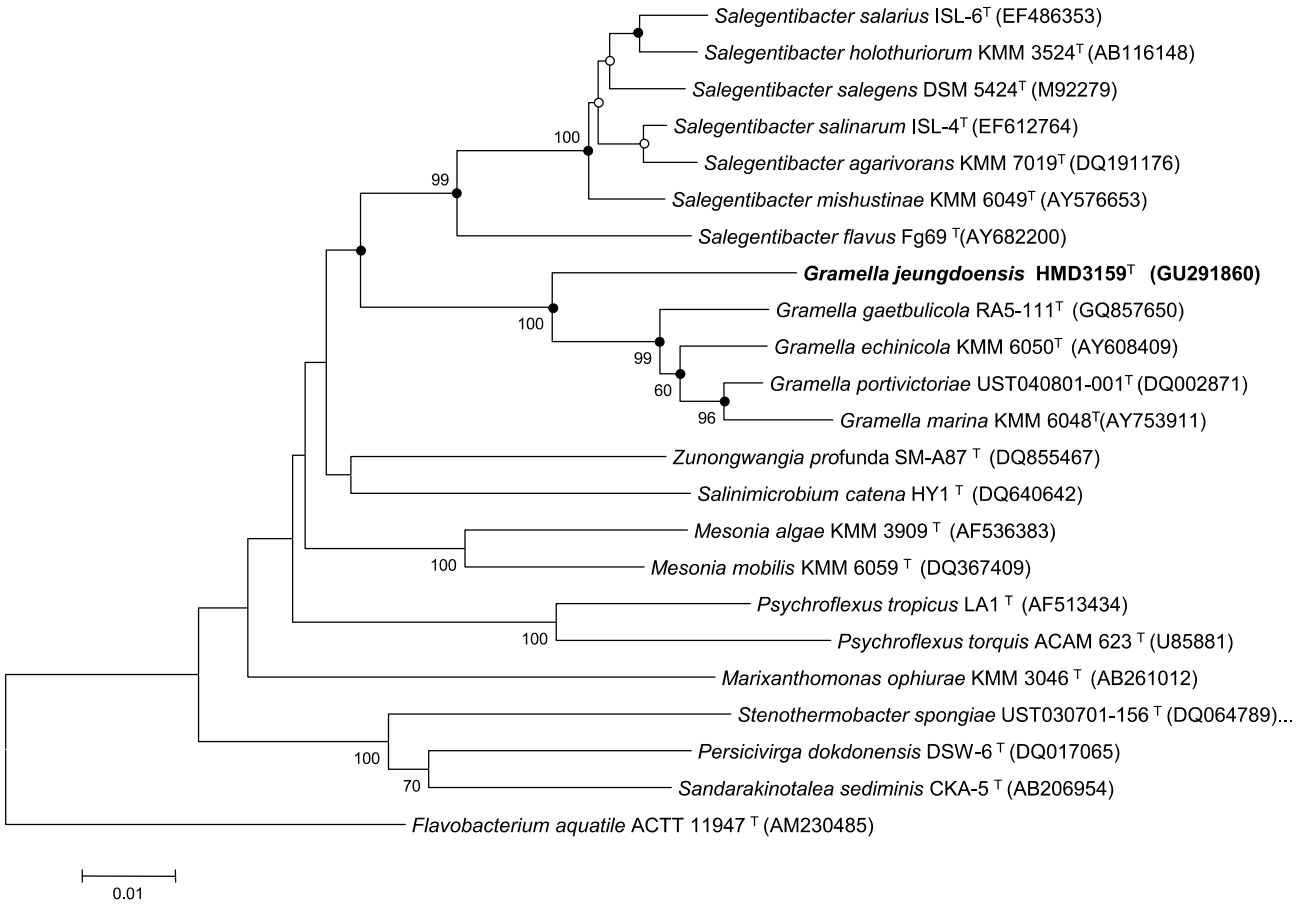

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the position of strain HMD3159^T in the genus *Gramella.* Bootstrap percentages (>50%) from both neighbour-joining are shown. Filled and open circles indicate nodes recovered by all three treeing methods or by two treeing methods, respectively. Bar, 0.01 substitution per nucleotide position.

1.0%) NaCl (w/v) was also tested in 1/10 strength marine broth (1:1; salt solution). Hydrolysis of casein [3.0% skimmed milk (Difco), v/v], crystalline cellulose (Whatman no. 1 filter paper, 1.0%) and starch (1.0%, w/v) were tested using modified MA (marine agar 27.6 g, agar 15 g per 1,000 ml distilled water) as the basal medium. Blood agar (Difco), MacConky agar (Difco), and tryptic soy agar (Difco) were used for growth and DNase test agar (Difco) was used for DNase assay. Other biochemical tests and carbon source oxidation tests were performed using API 20NE, API ZYM strips (bioMérieux, USA) and GN2 MicroPlates (Biolog, USA), according to the manufacturer's instructions except that suspensions for inoculation were prepared from bacteria grown in marine broth at 25°C for 3 days. The DNA G+C content was determined by using HPLC (Mesbah and Whitman, 1989). For fatty acid analysis, strain HMD3159^T and 3 references were grown on MA at 25°C for 3 days and the fatty acid methyl esters were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System. Isoprenoid quinones were isolated according to Minnikin *et al.* (1984) and analyzed by HPLC as described by Collins (1985).

Morphological, cultural, physiological and biochemical characteristics of strain HMD3159^T were listed in Table 1 and in the species description. The isolate exhibited a number of phenotypic similarities with respect to species of the genus *Gramella*, including cell morphology, yellow-colored pigments, no flexirubin-type pigments, growth of various NaCl concentrations and strictly aerobic growth. However, several characteristics of strain HMD3159^T clearly differentiated this strain from the type strains of *Gramella* species (Table 1). The fatty acid profile of strain HMD3159^T comprised iso-$C_{15:0}$ (26.3%), iso-$C_{17:0}$ 3OH (12.1%), iso-$C_{16:0}$(12.0%), summed feature 3 (comprising $C_{16:1}$ ω7*c* and/or $C_{16:1}$ ω6*c*; 11.0%), and summed feature 9 (iso-$C_{17:1}$ ω9*c* and/or 10-methyl $C_{16:0}$; 10.0%). This fatty acid profile was similar to those (iso-$C_{15:0}$, iso-$C_{17:0}$ 3OH, iso-$C_{16:0}$, and summed feature 3) of other *Gramella* species, although there were differences in the proportions of some fatty acids such as summed feature 9. Chemotaxonomic data confirm the phylogenetic affiliation of strain HMD3159^T as a member of the genus *Gramella*. The major respiratory quinone was MK-6. The DNA G+C content of strain HMD3159^T was 40.9 mol%, a similar range reported for members of genus *Gramella* (39.1-41.1 mol%). Therefore, strain HMD3159^T should be classified in the genus *Gramella* as a member of novel species, for which the name *Gramella jeungdonensis* sp. nov. is proposed.

Table 1. Differential characteristics of strain HMD3159^T and other phylogenetically-related members in the genus *Gramella*
Strains: 1, HMD3159^T; 2, *G. gaetbulicola* RA5-111^T; 3, *G. echinicola* KCTC 12278^T; 4, *G. portivictoriae* KCTC 22434^T; 5, *G. marina* KCTC 12336^T. Data of 1 to 5 were obtained from this study under the same experimental conditions. All the strains were grown under the same experimental conditions. +, positive; -, negative.

Characteristics	1	2	3	4	5
Growth at 42°C	+	+	-	-	-
Growth in 14% NaCl	+	-	+	-	+
API ZYM					
Trypsin	+	+	-	+	+
α-Chymotrypsin	-	+	+	+	+
β-Glucuronidase	+	-	-	-	-
N-Acetyl-β-glucosaminidase	+	+	-	-	-
Carbon utilization on GN2 microplate					
m-Inositol	+	-	-	+	-
Lactulose	+	-	-	+	+
D-Mannitol	-	-	+	+	-
β-Methyl-D-glucoside	+	-	+	-	+
D-Psicose	+	+	+	-	+
L-Rhamnose	+	-	-	+	+
Sucrose	+	+	+	-	+
D-Trehalose	+	+	+	-	+
Turanose	+	-	+	-	+
Acetic acid	+	+	+	-	-
D-Galactonic acid lactone	+	-	+	-	-
D-Glucuronic acid	+	+	+	-	-
Itaconic acid	+	-	+	-	+
α-Keto butyric acid	+	+	+	-	+
α-Keto valeric acid	+	+	-	+	+
Malonic acid	+	-	-	+	-
D-Saccharic acid	+	-	-	+	+
Succinamic acid	+	+	-	+	+
Glucuronamide	+	-	-	+	+
L-Alaninamide	+	-	-	+	+
L-Alanine	-	+	+	+	+
L-Aspartic acid	-	+	+	+	-
L-Glutamic acid	+	+	-	+	+
D,L-Carnitine	+	-	+	-	-
DNA G+C content (mol%)	40.9	39.1	41.1	41.1	40.0

Description of *Gramella jeungdoensis* sp. nov.

Gramella jeungdoensis (*jeung do nen' sis*. N.L. fem. adj. *jeungdoensis,* of or belonging to jeungdo, Korea*,* from where the type strain was isolated)

Cells are Gram-staining-negative, aerobic, rod shape, 0.4-0.6 μm in diameter and 0.8-1.0 μm in length. Colonies on MA are convex yellow, round and approximately 1 mm in diameter after 3 days at 25°C. Growth occurs on MA. No growth occurs on Blood agar, DNase test agar, MacConkey agar and TSA agar and no hydrolysis of casein and cellulose. But no growth occurs on starch. Growth occurs in the presence of 0.5-14% (w/v) NaCl (optimum, 4%), at pH 6-9 (optimum, pH 7), and at 15-42°C (optimum, 25°C). Oxidase, catalase activities, and esculin hydrolysis activity are present, but arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, and L-phenylalanine deaminase activities are absent. In the API ZYM gallery, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-glucuronidase, α-glucosidase, and *N*-acetyl-β-glucosaminidase activies are present. But lipase (C14), α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucosidase, α-mannosidase, and α-fucosidase activities are absent. The following compounds are utilized as sole carbon sources in GN2 MicroPlates: acetic acid, adonitol, cis-aconitic acid, citric acid, D,L-carnitine, D,L-lactic acid, D-galactonic acid, lactone, D-galactose, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, D-mannose, D-melibiose, D-psicose, D-raffinose, D-saccharic acid, D-sorbitol, D-trehalose, formic acid, gentiobiose, glucuronamide, glycogen, glycyl-L-aspartic acid, itaconic acid, lactulose, L-alaninamide, L-glutamic acid, L-leucine, L-proline, L-rhamnose, malonic acid, maltose, *m*-inositol, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, *p*-hydroxy phenlyacetic acid, propionic acid, succinamic acid, succinic acid mono-methyl-ester, sucrose, turanose, α-D-glucose, α-D-lactose, α-keto butyric acid, α-keto glutaric acid, α-keto valeric acid, and β-methyl-D-glucoside. The following carbon sources are not utilized:

Table 2. Cellular fatty acid composition (%) of strain HMD3159^T and other phylogenetically-related members in the genus *Gramella* Strains:1, HMD3159^T; 2, *G. gaetbulicola* RA5-111^T ; 3, *G. echinicola* KCTC 12278^T; 4, *G. portivictoriae* KCTC 22434^T; 5, *G. marina* KCTC 12336^T. Data of 1 to 5 were obtained from this study under the same experimental conditions. All the strains were grown under the same growth conditions (MA, 25°C, 3 days of incubation). Only fatty acids amounting to at least 1.0% of the total cellular fatty acids for at least one of the strains were shown. -, less than 1.0% of the total or not detected.

Fatty acids	1	2	3	4	5
iso-$C_{14:0}$	2.2	-	1.7	1.5	2.5
iso-$C_{15:0}$ G	1.4	-	1.3	-	-
iso-$C_{15:0}$	26.3	21.4	17.7	19.7	18.3
anteiso-$C_{15:0}$	4.4	4.0	8.0	9.1	14.2
$C_{15:1\ \omega}6c$	-	-	1.3	1.2	2.8
iso-$C_{16:1}$ H	4.6	-	4.1	2.2	3.6
iso-$C_{16:0}$	12.0	1.5	11.9	4.3	7.41
$C_{16:0}$	1.5	5.8	4.3	4.4	-
iso-$C_{15:0}$ 3OH	2.4	3.2	1.7	2.0	1.4
$C_{15:0}$ 2OH	-	1.5	2.3	3.4	4.4
anteiso-$C_{17:1}$ $\omega 9c$	-	-	2.6	2.7	2.9
$C_{17:1}$ $\omega 6c$	-	-	-	-	4.0
iso-$C_{16:0}$ 3OH	4.1	2.6	5.0	4.5	6.2
$C_{16:0}$ 3OH	-	-	1.5	1.3	1.1
iso-$C_{17:0}$ 3OH	12.1	22.2	9.7	15.9	8.3
$C_{17:0}$ 2OH	1.27	-	3.3	6.4	6.2
iso $C_{18:0}$	-	3.7	-	-	-
Summed Feature 1[a]	-	-	-	1.2	-
Summed Feature 3	11.0	16.7	15.4	13.0	8.3
Summed Feature 9	10.0	-	3.3	4.0	2.7

[a] Summed features represent groups of two or three fatty acids that could not be separated using the MIDI system. Summed feature 1, $C_{13:0}$ 3OH and /or iso-$C_{15:1}$ H; summed feature 3, $C_{16:1}$ $\omega 7c$ and/or $C_{16:1}$ $\omega 6c$; summed feature 9, iso-$C_{17:1}$ $\omega 9c$ and/or 10-methyl $C_{16:0}$.

α-cyclodextrin, 2,3-butanediol, 2-aminoethanol, bromosuccinic acid, D,L,α-glycerol phosphate, D-alanine, D-arabitol, D-cellobiose, dextrin, D-fructose, D-galacturonic acid, D-glucose-6-phosphate, D-mannitol, D-serine, glycerol, glycyl-L-glutamic acid, hydroxy-L-proline, i-erythritol, inosine, L-alanine, L-alanyl-glycine, L-arabinose, L-asparagine, L-aspartic acid, L-fucose, L-histidine, L-ornithine, L-phenylalanine, L-pyroglutamic acid, L-serine, L-threonine, phenylethyl-amine, putrescine, pyruvic acid methyl ester, quinic acid, sebacic acid, succinic acid, thymidine, Tween 40, Tween 80, uridine, urocanic acid, xylitol, α-hydroxybutyric acid, α-D-glucose-1-phosphate, β-hydroxybutyric acid, γ-hydroxybutyric acid, and γ-amino butyric acid. The major fatty acids are iso-$C_{15:0}$, iso-$C_{17:0}$ 3OH, iso-$C_{16:0}$, summed feature 3 (comprising $C_{16:1}$ $\omega 7c$ and/or $C_{16:1}$ $\omega 6c$) and summed feature 9 (iso-$C_{17:1}$ $\omega 9c$ and/or 10-methyl $C_{16:0}$). The complete fatty acid content is given in Table 2. The DNA G+C content is 40.9 mol%.

The type strain, HMD3159^T (=KCTC 32123^T =CECT 7683^T), was isolated from solar saltern form Jeungdo, Jeolnam, Republic of Korea.

The GenBank accession number for the 16S rRNA gene sequence of strain HMD3159^T is GU291860.

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