Joostella marina gen. nov., sp. nov., a novel member of the family *Flavobacteriaceae* isolated from the East Sea

Zhe-Xue Quan,[1,2] Yi-Ping Xiao,[1] Seong Woon Roh,[2] Young-Do Nam,[2] Ho-Won Chang,[2] Kee-Sun Shin,[2] Sung-Keun Rhee,[3] Yong-Ha Park[2] and Jin-Woo Bae[2,4]

Correspondence
Jin-Woo Bae
baejw@kribb.re.kr

[1]Department of Microbiology and Microbial Engineering, School of Life Sciences, Fudan University, Shanghai 200433, PR China

[2]Biological Resource Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon 305-333, Republic of Korea

[3]Department of Microbiology and Biotechnology Research Institute, Chungbuk National University, Cheongju 361-763, Republic of Korea

[4]Environmental Biotechnology National Core Research Center, Gyeongsang National University, Jinju 660-701, Republic of Korea

A Gram-negative, non-spore-forming, non-motile, yellow-pigmented, strictly aerobic bacterial strain, designated En5^T, was isolated from the East Sea of Korea and was subjected to a polyphasic taxonomy study. Strain En5^T grew optimally at 30 °C, in the presence of 1–3 % (w/v) NaCl and at pH 5.3–7.6. The major respiratory lipoquinone was MK-6 and the major fatty acids were iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH and iso-$C_{17:1}\omega9c$. The DNA G+C content of strain En5^T was 30.1 mol%. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain En5^T formed a distinct evolutionary lineage within the family *Flavobacteriaceae* and shared 93 % sequence similarity with the type strains of both *Galbibacter mesophilus* and *Zhouia amylolytica*. On the basis of its phenotypic and phylogenetic properties, strain En5^T is suggested to represent a novel species of a new genus in the family *Flavobacteriaceae*, for which the name *Joostella marina* gen. nov., sp. nov. is proposed. The type strain is En5^T (=KCTC 12518^T=DSM 19592^T=CGMCC 1.6973^T).

The family *Flavobacteriaceae*, one of the major branches of the phylum *Bacteroidetes*, was first proposed by Jooste (1985), and was included in the first edition of *Bergey's Manual of Systematic Bacteriology* (Reichenbach, 1989). The name of the family was subsequently validated (Reichenbach, 1992), and its description was subsequently emended considerably (Bernardet *et al.*, 1996, 2002). Many new organisms have been allocated to the family over the past few years (Bernardet & Nakagawa, 2006). Within the single year 2006, 13 new genera and 20 novel species were described in the family *Flavobacteriaceae*; all except *Cloacibacterium normanense* (Allen *et al.*, 2006) were isolated from marine environments.

Strain En5^T was isolated from coastal seawater in the East Sea of Korea at a depth of 100 m by the dilution-plating technique on marine agar 2216 (MA; Difco). Cell biomass for DNA extraction and for fatty acid methyl ester and quinone analyses was obtained from MA plates after 3 days incubation at 30 °C. Cell morphology was examined by light microscopy (Nikon) and transmission electron microscopy (EM912Ω; Leo Zeiss) after negative staining with 1 % (w/v) phosphotungstic acid. Gliding motility and flexirubin-type pigments were investigated according to the methods specified by Bernardet *et al.* (2002). The Gram reaction was determined by using cells grown on MA at 30 °C for 24 h, according to the method described by Gerhardt *et al.* (1994). Catalase activity was investigated via bubble production in 3 % (v/v) hydrogen peroxide solution; oxidase activity was determined by oxidation of 1 % (w/v) tetramethyl *p*-phenylenediamine (Merck). Hydrolysis of starch and Tween 80, acid production from cellobiose, utilization of sucrose and endospore formation were determined as described by Dong & Cai (2001) by using media prepared with artificial seawater (per litre distilled water: 23.5 g NaCl, 5.0 g $MgCl_2.6H_2O$, 3.9 g Na_2SO_4, 1.1 g $CaCl_2$, 0.7 g KCl, 0.2 g $NaHCO_3$, 0.01 g KBr, 0.03 g H_3BO_3). Hydrolysis of agar was tested on

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain En5^T is EF660761.

marine broth (MB; Difco) solidified with 1.5 % agar. Additional enzyme activities, acid production from carbohydrates and substrate utilization as sole carbon source were determined by using API ZYM and API 20NE galleries (bioMérieux) according to the manufacturer's instructions. Growth in the presence of 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 15, 18 and 20 % (w/v) NaCl was tested in R2A broth (Reasoner & Geldreich, 1985). Growth at pH 3.1, 4.1, 5.3, 6.9, 7.6, 8.8, 9.3, 10.5 and 11.8 was tested in MB, and growth at 4, 10, 20, 25, 30, 37, 42 and 45 °C was tested on MA. Growth on MacConkey agar was also determined. Anaerobic growth was determined on MA in an anaerobic test tube by using the AnaeroGen kit (Oxoid). The cultural, physiological and biochemical characteristics of strain En5^T are given in the species description and in Table 1.

Chromosomal DNA was isolated and purified by using a Cell Culture DNA Midi kit (Qiagen) according to the manufacturer's protocol. Respiratory lipoquinones were analysed by reversed-phase HPLC as described by Komagata & Suzuki (1987). For quantitative analysis of the cellular fatty acid composition, a loopful of cell mass was harvested and the cellular fatty acids were extracted, saponified and methylated according to the protocol of the Sherlock Microbial Identification System (MIDI). The fatty acids were analysed by GC (Hewlett Packard 6890) and identified by using the Microbial Identification software package (Sasser, 1990). The G+C content of the DNA of strain En5^T was determined by using the fluorescence monitoring method (Xu *et al.*, 2000; Gonzalez & Saiz-Jimenez, 2002) with a LightCycler (Roche Diagnostics). The DNA of *Escherichia coli* B (Sigma-Aldrich) was used as the calibration reference.

The major respiratory quinone of strain En5^T was menaquinone 6 (MK-6). The cellular fatty acid profiles of strain En5^T and related genera are presented in Table 2. The major components in strain En5^T were branched fatty acids such as iso-$C_{15:0}$ (14.5 %), iso-$C_{17:0}$ 3-OH (13.3 %) and iso-$C_{17:1}\omega9c$ (12.5 %). Strain En5^T also contained 12.3 % of a characteristic unknown fatty acid with an equivalent chain length (ECL) of 13.566. The DNA G+C content of strain En5^T was 30.1 mol%.

The 16S rRNA gene of strain En5^T was amplified by PCR by using the universal primer pair 9F and 1512R as described by Quan *et al.* (2005). The PCR product was purified with a QIAquick PCR purification kit (Qiagen)

Table 1. Differential characteristics of strain En5^T and the type strains of related taxa in the family *Flavobacteriaceae*

Strain: 1, En5^T; 2, *Galbibacter mesophilus* Mok-17^T (Khan *et al.*, 2007); 3, *Zhouia amylolytica* HN-171^T (Liu *et al.*, 2006). All strains are positive for the following characteristics: production of yellow pigments; presence of catalase and oxidase activities; hydrolysis of starch; and utilization of D-glucose, D-mannose and sucrose as sole carbon sources. All strains are negative for the following characteristics: production of flexirubin-type pigments; gliding motility; growth at 4 °C; presence of urease activity; hydrolysis of agar; and acid production from D-glucose. +, Positive; −, negative; W, weakly positive; ND, not determined.

Characteristic	1	2	3
Growth with 15 % NaCl	+	−	−
Optimal NaCl concentration for growth (%)	1–3	3–5	4.5–5.0
Growth at 42 °C	−	+	+
Optimal growth temperature (°C)	30	25–30	30
Hydrolysis of:			
Casein	−	W	−
Gelatin	−	+	+
Tween 80	+	ND	−
Nitrate reduction	−	+	−
DNA G+C content (mol%)	30.1	37	34.5

Table 2. Fatty acid content (%) of strain En5^T and the type strains of related taxa in the family *Flavobacteriaceae*

Strain: 1, En5^T; 2, *Galbibacter mesophilus* Mok-17^T (Khan *et al.*, 2007); 3, *Zhouia amylolytica* HN-171^T (Liu *et al.*, 2006). The strains studied were grown under different culture conditions. ND, Not detected; tr, trace (<1 %). Fatty acids amounting to <1 % in all strains studied are not listed.

Fatty acid	1	2	3
Straight-chain saturated			
$C_{14:0}$	ND	ND	2.5
$C_{15:0}$	5.0	ND	9.4
$C_{16:0}$	2.0	tr	4.9
$C_{15:0}$ 2-OH	tr	ND	1.4
$C_{16:0}$ 3-OH	1.4	ND	ND
Branched saturated			
iso-$C_{15:0}$	14.5	16.0	14.9
iso-$C_{16:0}$	2.5	tr	ND
iso-$C_{13:0}$ 3-OH	ND	ND	1.4
iso-$C_{15:0}$ 3-OH	3.2	7.0	3.9
iso-$C_{16:0}$ 3-OH	1.3	tr	ND
iso-$C_{17:0}$ 3-OH	13.3	19.0	ND
anteiso-$C_{15:0}$	2.3	tr	1.1
Mono-unsaturated			
iso-$C_{15:1}$	9.8	17.0	24.2
iso-$C_{16:1}$	1.1	1.0	ND
$C_{15:1}\omega6c$	ND	3.0	ND
$C_{17:1}\omega6c$	1.0	2.0	ND
iso-$C_{17:1}\omega9c$	12.5	9.0	ND
Summed feature 3*	8.4	15.0	10.7
Summed feature 4*	2.2	2.0	ND
Unknown			
ECL 13.566†	12.3	ND	ND

*Summed feature 3 comprised $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH. Summed feature 4 comprised iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B.
†ECL 13.566, unidentified fatty acid with an equivalent chain length of 13.566.

and sequenced by using an Applied Biosystems 3730X1 DNA analyser and the primers 519F, 536R, 907F and 1100R (Quan *et al.*, 2005). 16S rRNA gene sequences of related taxa were obtained from GenBank and multiple alignments were performed by using the CLUSTAL_X program (Thompson *et al.*, 1997). Gaps at the 5′ and 3′ ends of the alignment were omitted from further analysis. Phylogenetic trees were constructed based on the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Swofford, 1993) algorithms by using the MEGA3 program (Kumar *et al.*, 2004) with bootstrap values based on 500 replications (Felsenstein, 1985). Evolutionary distances were calculated according to the method of Jukes & Cantor (1969).

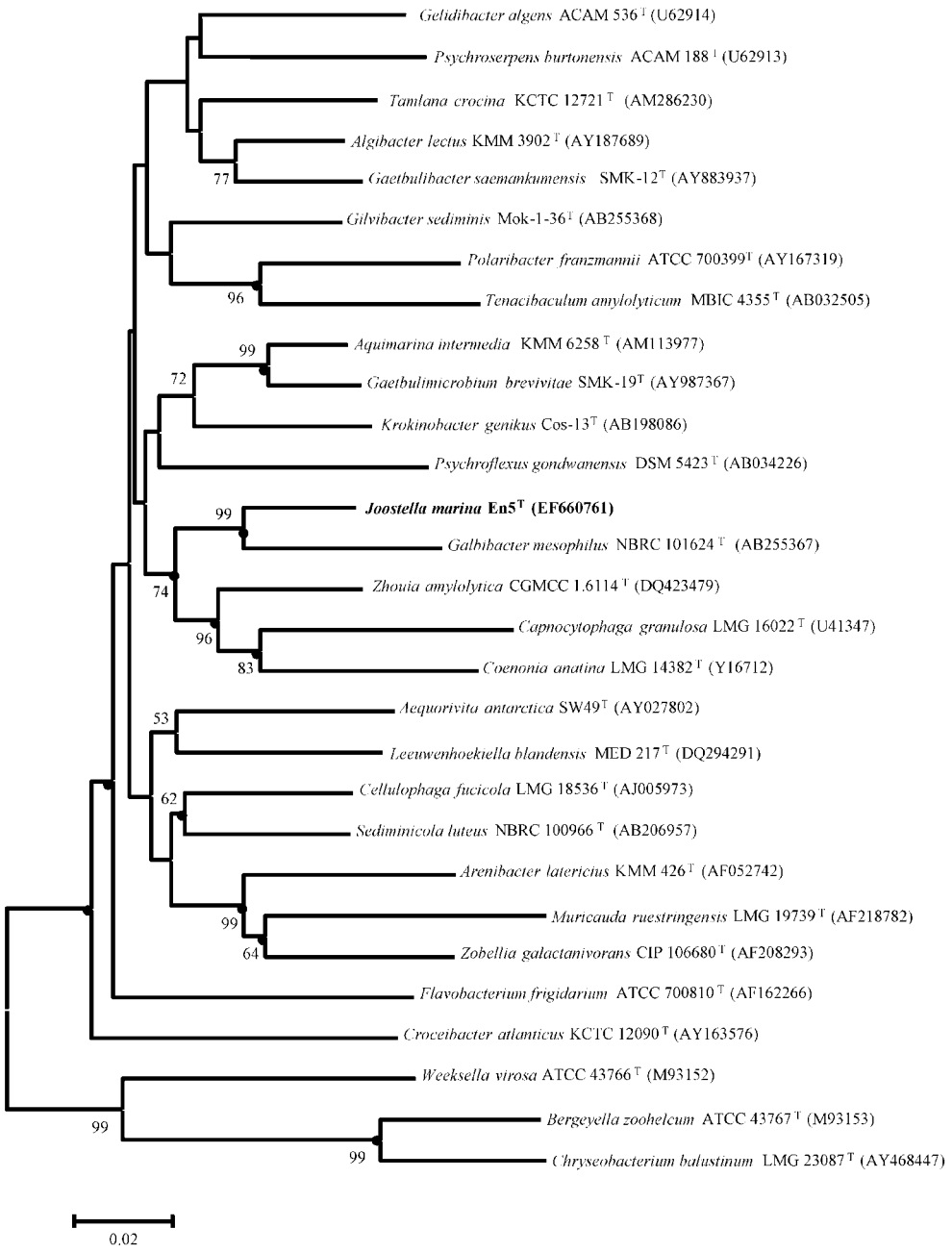

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between strain En5^T and related genera in the family *Flavobacteriaceae*. Closed circles indicate branches that were also recovered by using the maximum-parsimony algorithm. Bootstrap values (expressed as percentages of 1000 replications) of >50 % are shown at branch points. Bar, 2 substitutions per 100 nucleotide positions.

The nearly-complete 16S rRNA gene sequence of strain En5^T (1427 bp) was obtained. Preliminary sequence comparisons with 16S rRNA gene sequences deposited in the GenBank database indicated that strain En5^T belonged to the family *Flavobacteriaceae* in the phylum *Bacteroidetes*. On the basis of 16S rRNA gene sequence similarity, its closest relatives were *Galbibacter mesophilus* (Khan *et al.*, 2007) and *Zhouia amylolytica* (Liu *et al.*, 2006), the type strains of these two species both sharing 93 % sequence similarity with strain En5^T. In the neighbour-joining phylogenetic tree (Fig. 1) based on 16S rRNA gene sequences, strain En5^T formed a monophyletic clade with *G. mesophilus* and *Z. amylolytica* that was supported by a high bootstrap value. The topology of the maximum-parsimony tree was essentially the same (data not shown).

On the basis of the large phylogenetic distance and differential phenotypic characteristics with its closest relatives, we suggest that strain En5^T represents a novel species of a new genus in the family *Flavobacteriaceae*, for which the name *Joostella marina* gen. nov., sp. nov. is proposed.

Description of *Joostella* gen. nov.

Joostella (Jo.o.stel′la. N.L. fem. n. *Joostella* named after Professor P. J. Jooste, who first proposed the family *Flavobacteriaceae*).

Cells are Gram-negative, aerobic, non-spore-forming, non-motile rods. Oxidase- and catalase-positive. Major fatty acids are iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH and iso-$C_{17:1}\omega9c$; the major respiratory quinone is MK-6. The DNA G+C content of the type species is about 30 mol%. Member of the family *Flavobacteriaceae*. The type species is *Joostella marina*.

Description of *Joostella marina* sp. nov.

Joostella marina (ma.ri′na. L. fem. adj. *marina* belonging to the sea, marine).

The description is as for the genus with the following additional properties. Cells are usually 0.20–0.30 μm wide and 1.0–2.0 μm long. Good growth occurs on R2A agar and MA. Colonies are translucent and shiny with entire edges, becoming mucoid after 3 days incubation. Bright-yellow non-flexirubin-type pigments are produced. Growth occurs at 10–37 °C (optimum, 30 °C), but not at 4 or 42 °C. Growth occurs at pH 5.3–10.5 (optimum, pH 5.3–7.6). Growth occurs in the presence of 0–15 % NaCl (optimum, 1–3 %), but not in the presence of 18 % NaCl. Positive for α-glucosidase, β-glucosidase, β-galactosidase, α-mannosidase, alkaline phosphatase, acid phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, naphthol-AS-BI-phosphohydrolase and *N*-acetyl-β-glucosaminidase activities, but negative for urease, arginine dihydrolase, lipase (C14), α-chymotrypsin, α-galactosidase, β-glucuronidase and α-fucosidase activities. Acid is produced from cellobiose, but not from glucose. Starch, aesculin and Tween 80 are hydrolysed, but agar, casein and gelatin are not. Indole is not produced. Nitrate and nitrite are not reduced. The following substrates are utilized as sole carbon source: glucose, sucrose, arabinose, mannose and maltose. The following substrates are not utilized as sole carbon source: mannitol, *N*-acetylglucosamine, gluconate, caprate, adipate, malate, citrate and phenylacetate. The major fatty acids are iso-$C_{15:0}$ (14.5 %), iso-$C_{17:0}$ 3-OH (13.3 %) and iso-$C_{17:1}\omega9c$ (12.5 %). The G+C content of the genomic DNA is 30.1 mol%.

The type strain, En5^T (=KCTC 12518^T=DSM 19592^T=CGMCC 1.6973^T), was isolated from seawater in the East Sea of Korea, at a depth of 100 m.

Acknowledgements

This study was supported by the KRIBB Research Initiative Program and Environmental Biotechnology National Core Research Center (KOSEF: R15-2003-012-02002-0), Ministry of Science and Technology (MOST), Republic of Korea.

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