Yeosuana aromativorans gen. nov., sp. nov., a mesophilic marine bacterium belonging to the family *Flavobacteriaceae*, isolated from estuarine sediment of the South Sea, Korea

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A marine bacterium, GW1-1^T, capable of degrading benzo[a]pyrene (BaP), was isolated from estuarine sediments of the South Sea (the Korea Strait), Korea, after an enrichment culture maintained for 2 years in a medium supplemented with a mixture of BaP and pyrene. The strain formed yellowish-brown colonies on marine agar 2216. Cells were strictly aerobic, non-motile, Gram-negative rods and produced non-diffusible carotenoid pigments. Optimal growth occurred in the presence of 1 % (w/v) NaCl and at pH 7 and 33–36 °C. No growth occurred without supplementation with either CaCl₂ or MgCl₂, even in the presence of NaCl. Phylogenetic analysis based on the nearly complete sequence of the 16S rRNA gene revealed that the isolate formed a phyletic lineage with the genera *Gelidibacter* (93.9–94.7 % gene sequence similarity), *Subsaximicrobium* (93.3 %) and *Subsaxibacter* (93.9 %). The isolate also showed high sequence similarities to *Gaetbulibacter saemankumensis* (94.5 %), *Algibacter lectus* (94.2 %), members of the genus *Bizionia* (93.6–94.3 %) and *Formosa algae* (93.2 %), even though it belonged to a different phyletic line. The major respiratory quinones of the isolate were menaquinones MK-5 and MK-6. The DNA G + C content was 51.4 mol%. Dominant fatty acids were i-15 : 0, a-15 : 0, i-15 : 1 ω 10c and 16 : 1. On the basis of this polyphasic taxonomic evidence, strain GW1-1^T is classified as a member of a novel genus and species in the family *Flavobacteriaceae*, for which the name *Yeosuana aromativorans* gen. nov., sp. nov. is proposed. The type strain of the type species is GW1-1^T (=KCCM 42019^T=JCM 12862^T).

The family *Flavobacteriaceae* (Reichenbach, 1989; Bernardet *et al.*, 2002) is one of the major phylogenetic lineages within the phylum *Bacteroidetes* (Garrity & Holt, 2001). The family currently consists of more than 40 genera with validly published names and several more proposed genera (<http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi>). Many members of the family have been isolated from sea water and show psychrophilic or psychrotolerant characteristics (Madigan *et al.*, 2003). In recent years, a number of strains belonging to this group and showing mesophilic

characteristics, such as *Gelidibacter mesophilus* (Macián *et al.*, 2002) and *Gaetbulibacter saemankumensis* (Jung *et al.*, 2005), have been isolated from tropical areas. These reports show that members of the family *Flavobacteriaceae* isolated from tropical areas may display mesophilic characteristics.

In the present study, we describe a novel marine bacterium, GW1-1^T, isolated from a long-term enrichment culture of estuarine sediments with a mixture of benzo[a]pyrene (BaP) and pyrene. The removal of these potent carcinogenic compounds from natural environments is a matter of great concern (Juhász & Naidu, 2000). Although phylogenetically related to members of the genus *Gelidibacter*, strain GW1-1^T shows many different physiological and chemotaxonomic characteristics. On the basis of evidence from polyphasic studies, strain GW1-1^T is proposed as a member of a novel genus and species in the family *Flavobacteriaceae*.

Strain GW1-1^T was isolated from estuarine sediments of Gwangyang Bay, Yeosu City, South Sea (the Korea Strait), Republic of Korea. The procedures used to isolate the strain

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Abbreviation: BaP, Benzo[a]pyrene.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain GW1-1^T is AY682382.

Graphs showing the degradation rates of BaP by strain GW1-1^T and HPLC analysis of the major respiratory quinones and an additional phylogenetic tree are available as supplementary figures in IJSEM Online.

Table 1. Phenotypic characteristics that differentiate strain GW1-1^T from closely related members of the family *Flavobacteriaceae*

Taxa: 1, GW1-1^T; 2, *Gelidibacter*; 3, *Algibacter lectus* KMM 3902^T; 4, *Bizionia*; 5, *Formosa algae* KMM 3553^T; 6, *Gaetbulibacter saemankumensis*; 7, *Subsaxibacter broadyi* P7^T; 8, *Subsaximicrobium*. Data for reference taxa are from Bowman *et al.* (1997), Macián *et al.* (2002), Ivanova *et al.* (2004), Nedashkovskaya *et al.* (2004, 2005), Bowman & Nichols (2005) and Jung *et al.* (2005). A requirement for sea salts indicates that Na⁺ alone does not support growth; instead, the strain requires additional cations for growth, such as Mg²⁺ and Ca²⁺, present in sea water. For taxa 2, 4, 6 and 8, values are characteristics for all species in the genus or for all strains in the species. +, Positive; −, negative; ND, not determined; v, variable.

Characteristic	1	2	3	4	5	6	7	8
Cell size (µm)	0.7–1.7 × 0.2–0.3	1–8 × 0.3–0.6	2–3 × 0.4–0.5	1–5 × 0.3–0.5	0.8–1.8 × 0.4–0.9	0.4–0.5 × 3.0–4.5	0.3–0.5 × 0.4–1.0	0.3–0.3 × 1–6
Colony size (mm)	1–1.5	2–3	3–4	2–4	1–3	ND	<1	ND
Gliding motility	–	+	+	–	+	+	+	+
Temperature range for growth (°C)*	23–39 (33–36)	0–37 (15–18)	4–35 (21–23)	4–36 (23–25)	5–35 (23)	13–40 (25–30)	–2 to 20	–2 to 25
pH range for growth*	5–8 (7)	ND	ND	ND	5.0–10.0 (8.0–8.5)	5.5–ND (7.0–8.0)	ND	ND
NaCl concentration for growth (% w/v)*	0.5–4.0 (2.0)	0–15	1–6	1–17.6	0–6	0–7 (2–5)	0.5–7.3 (1.8–2.3)	0.5–12
Requirement for:								
Oxygen	+	+	–	+	–	–	+	+
Sea salts	+	–	–	–	–	–	–	–
Yeast extract	–	–	–	+	+	–	+	–
Oxidase/catalase activity	–/+	–/+	+/+	+/+	+/+	+/+	ND/+	ND/+
Nitrate reduction	–	–	–	–	+	+	–	–
Production of:								
H ₂ S	–	–	–	+	–	–	–	–
Indole	–	–	–	–	–	–	–	–
Urease	–	–	–	–	+	–	–	–
Acid from carbohydrates	–	+	+	–	+	+	–	–
Hydrolysis of:								
Agar	–	–	+	–	–	ND	–	–
Casein	–	v	–	+	–	–	–	+
Gelatin	+	v	+	+	+	–	+	+
Starch	–	v	v	+	+	+	–	+
DNA G+C content (mol%)	51.4	36–42	31–33	38–45	34–35	34.7–34.9	35–36	38–41
Isoprenoid quinone(s)	MK-5, MK-6	MK-6	MK-6	MK-6	ND	MK-6	MK-6	MK-6

*Optimum range shown in parentheses.

and to study BaP degradation were as previously described by Kwon *et al.* (2005) and Zhang *et al.* (2004). Among the different colonies that grew on marine agar 2216 (MA; Difco) after a 2-year enrichment culture in minimal liquid medium MM2 supplemented with a mixture of BaP and pyrene, a tiny, yellowish-brown colony was isolated and named GW1-1^T. The isolate was cultivated on MA for morphological and biochemical characterization.

The fluorescence of BaP in culture medium inoculated with strain GW1-1^T was significantly decreased during incubation compared with that of a non-inoculated control, indicating that the isolate is capable of degrading BaP (Zhang *et al.*, 2004; see Supplementary Fig. S1 in IJSEM Online).

Unless otherwise stated, physiological and morphological characterization was conducted according to Sohn *et al.* (2004) and Kwon *et al.* (2005). Bacterial suspensions used to inoculate API 20NE (bioMérieux) and Microlog GN2 systems (Biolog) were prepared in 1% sea salts (Sigma) solution. Degradation of starch and casein and production

of hydrogen sulfide were tested by the methods of Smibert & Krieg (1994). The physiological, biochemical and morphological characteristics of strain GW1-1^T are given in the genus and species descriptions and in Table 1.

The cellular fatty acid profile of the strain was determined according to Sohn *et al.* (2004). Dominant fatty acids of strain GW1-1^T were i-15:0 (21.7%), a-15:0 (14.9%), i-15:1 ω 10c (14.8%) and 16:1 (10.9%). The fatty acid profile of strain GW1-1^T is compared with those of closely related genera of the family *Flavobacteriaceae* in Table 2.

The major respiratory quinones, MK-5 and MK-6, were determined by HPLC analysis according to Collins (1985). All genera belonging to the family *Flavobacteriaceae* have been reported to contain MK-6 as a major respiratory quinone. Strikingly, the amount of MK-5 in strain GW1-1^T is comparable to that of MK-6 as the area ratio of MK-5 to MK-6 is approximately 71% (see Supplementary Fig. S2 in IJSEM Online). The DNA G+C content of strain GW1-1^T was 51.4 mol%, as determined by HPLC using a symmetry

Table 2. Cellular fatty acid content (%) of strain GW1-1^T and closely related members of the family *Flavobacteriaceae*

Taxa: 1, GW1-1^T; 2, *Gelidibacter*; 3, *Algibacter lectus* KMM 3902^T; 4, *Bizionia*; 5, *Formosa algae* KMM 3553^T; 6, *Gaetbulibacter saemankumensis*; 7, *Subsaxibacter broadyi* P7^T; 8, *Subsaximicrobium*. Data for reference taxa are from Bowman *et al.* (1997), Macián *et al.* (2002), Ivanova *et al.* (2004), Nedashkovskaya *et al.* (2004, 2005), Bowman & Nichols (2005) and Jung *et al.* (2005). Fatty acid percentages of less than 3% of the total fatty acids are not shown. For taxa 2, 4, 6 and 8, values shown are the range of percentages for all species in the genus or for all strains of the species. br, Branched fatty acids including iso- and anteiso- forms; tr, trace.

Fatty acid	1	2	3	4	5	6	7	8
15:0	5.3	2.4–5.3	13.4		27.2			
16:0				2.3–4.3				
18:0				1.2–3.4				
i13:0				tr–3.0				
i15:0	21.7	3.4–8.8	12.5	3.1–13.2	13.3	23.0	6.3–6.4	7.4–10.4
a15:0	14.9	10.5–17.7	7.2	10.1–20.8	7.2	10.8	14.3–14.5	9.9–17.1
i15:1 ω 10c	14.8	5.3–11.4	13.4	2.1–12.5	15.3	12.5	6.2–6.7	6.7–13.4
a15:1 ω 10c		11.8–16.6		5.8–14.0			9.9–15.5	5.9–14.7
i16:0		1.4–4.4		1.3–5.8				
br 16:1		1.4–10.3		1.2–5.4			3.5–7.7	1.9–4.7
a17:0	4.2							
i17:1 ω 7c				1.7–15.1				3.5–5.1
a17:1 ω 7c		1.9–3.4		1.5–4.6			4.0–7.4	2.1–3.8
15:1 ^{a*}		2.7–4.2	10.9	1.2–2.3	14.0			
16:1 ^b	10.9	7.4		3.0–6.3	3.3		9.4–14.3	9.5–13.9
17:1 ^c		3.7						
18:1							8.2–18.7	
i15:0 3-OH		2.2–6.2	9.4	3.4–32.7		8.2	7.1–8.0	9.0–11.9
i16:0 3-OH		4.1–12.2		1.3–8.5		2.0	5.0–6.0	8.8–13.7
i17:0 3-OH		0–3.1	9.1	0.5–4.3		15.2	2.3–3.2	6.1–6.3
10-Methyl 16:0	4.7							
17:0 cyclo					7.4			
Summed feature 3 ^d						10.4		

*Contains one or more of the following fatty acids: ^a15:1 ω 4c, 15:1 ω 6c and/or 15:1 ω 11c; ^b16:1 ω 5c, 16:1 ω 7c, 16:1 ω 9c and/or 16:1 ω 13c; ^c17:1 ω 6c and/or 17:1 ω 8c; ^d16:1 ω 7c and/or i-15:0 2-OH.

reversed-phase C18 column (Waters) (Stackebrandt & Liesack, 1993). As shown in Table 1, the DNA G+C content of strain GW1-1^T is surprisingly higher than that of closely related taxa.

Extraction of genomic DNA and amplification of the 16S rRNA gene were conducted according to Sohn *et al.* (2004). A phylogenetic tree of strain GW1-1^T and members of closely related genera was generated based on the maximum-likelihood distance model and the neighbour-joining method. 16S rRNA gene sequences of *Bacteroides fragilis* ATCC 25285^T (GenBank no. NC_003228) and *Sphingobacterium spiritivorum* DSM 2582 (AJ459411) served as outgroups. Finally, 1304 unambiguously aligned sequences were compared. The closest neighbours of strain GW1-1^T are *Gelidibacter algens* (94.7%), *Gaetbulibacter saemankumensis* (94.5%) and *Gelidibacter gilvus* (94.4%). Phylogenetic analysis of 16S rRNA gene sequences from organisms with validly published names revealed that strain GW1-1^T shared the phyletic line grouping the genera *Gelidibacter*, *Subsaxibacter* and *Subsaximicrobium* (Fig. 1). *Gaetbulibacter saemankumensis* occupied a different phyletic line together with *Algibacter lectus*, *Formosa algae* and members of the genus *Bizionia*. When sequences of unidentified strains were included in the phylogenetic analysis, isolate GW1-1^T shared a phyletic line with several strains isolated from the east

coast of the USA and tentatively identified as *Gelidibacter* sp. strains (Lydell *et al.*, 2004) and with one unidentified bacterium isolated from a commercial nitrifying inoculum (see Supplementary Fig. S3 in IJSEM Online).

The genus *Gelidibacter* (Bowman *et al.*, 1997) currently comprises four recognized species isolated from polar areas [*Gelidibacter algens* (Bowman *et al.*, 1997), *Gelidibacter gilvus* and *Gelidibacter salicanalis* (Bowman & Nichols, 2005)] or from Mediterranean sea water (*Gelidibacter mesophilus*; Macián *et al.*, 2002). In contrast to members of the genus *Gelidibacter*, optimal growth of strain GW1-1^T occurred at temperatures higher than 30 °C and poor growth was observed at below 20 °C, indicating that it is strictly mesophilic. Members of the recently reported genus *Gaetbulibacter* and of the genus *Formosa* also show mesophilic growth characteristics; however, their phylogenetic position based on 16S rRNA gene sequences and their DNA G+C contents clearly differentiate them from strain GW1-1^T. Possession of MK-5 as well as MK-6 as major respiratory quinones is also a feature that distinguishes strain GW1-1^T from other taxa in the family *Flavobacteriaceae*. Consequently, strain GW1-1^T should be classified as a member of a new genus and novel species in the family *Flavobacteriaceae*, for which the name *Yeosuana aromativorans* gen. nov., sp. nov. is proposed.

Fig. 1. Phylogenetic tree based on nearly complete 16S rRNA gene sequences (1304 unambiguously aligned base pairs) showing the relationship between strain GW1-1^T and other members of the family *Flavobacteriaceae*. The tree is based on the maximum-likelihood distance model and the neighbour-joining method. Bootstrap values >50% of 1000 resampled datasets are shown. Only species with validly published names were included in the phylogenetic analysis. An additional phylogenetic tree, including several unidentified organisms, is available as Supplementary Fig. S3 in IJSEM Online. Bar, 0.03 substitutions per nucleotide position.

Description of *Yeosuana* gen. nov.

Yeosuana (Yeo.su.a'na. N.L. fem. n. *Yeosuana* named after Yeosu City, where the type strain of the type species was isolated).

Cells are strictly aerobic, non-motile, Gram-negative rods. Yellowish-brown colonies are formed on marine agar 2216. No gliding motility. Produce non-diffusible carotenoid pigments, but flexirubin type pigments are absent. DNA G+C content is 51.4 mol%. Major respiratory quinones are MK-5 and MK-6. Major cellular fatty acids are straight chain and branched chain unsaturated fatty acids. Oxidase activity is absent and catalase activity is weak. As determined by 16S rRNA gene sequence analysis, the genus *Yeosuana* is a member of the family *Flavobacteriaceae*, phylum *Bacteroidetes*. The type species is *Yeosuana aromativorans*.

Description of *Yeosuana aromativorans* sp. nov.

Yeosuana aromativorans (a.ro.ma'ti.vo.rans. L. n. *aroma-tis* spice; L. part. adj. *vorans* devouring; N.L. part. adj. *aromativorans* degrading aromatic compounds).

Exhibits the following characteristics in addition to those described for the genus. Cells are 0.7–2.0 µm in length and 0.2–0.3 µm wide. Growth occurs between 23 and 39 °C (optimum temperature, 33–36 °C) at pH 5–8 (optimum, pH 7) and with 0.5–3.0 % NaCl (optimum, 1 %). In addition to NaCl, requires either 0.18 % (w/v) CaCl₂ or 0.59 % (w/v) MgCl₂ for growth. Tests positive for β-glucosidase, β-galactosidase and protease activities. Degrades α-cyclodextrin, dextrin, cellobiose, D-fructose, gentiobiose, α-D-glucose, α-D-lactose, maltose, D-mannose, sucrose, methyl pyruvate, α-ketobutyric acid, L-proline, glucose 1-phosphate and glucose 6-phosphate. Weakly utilizes D-galactose, α-ketoglutaric acid, L-alanine, L-alanyl glycine, L-asparagine, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, hydroxy-L-proline, uridine and L-threonine as sole carbon sources. Is capable of degrading polycyclic aromatic hydrocarbons including pyrene and BaP. Other phenotypic features are listed in Table 1. Dominant fatty acids are i-15:0 (21.7 %), a-15:0 (14.9 %), i-15:1ω10c (14.8 %) and 16:1 (10.9 %).

The type strain, GW1-1^T (= KCCM 42019^T = JCM 12862^T), was isolated from estuarine sediment of Gwangyang Bay, Yeosu City, Korea.

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