Pontirhabdus pectinivorans gen. nov., sp. nov., isolated from seawater

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A yellow-coloured, rod-shaped, Gram-reaction-negative and aerobic bacterial strain, designated JC2675^T, was isolated from a seawater sample from Jeju Island, Korea. The isolate required sea salts for growth. Gliding motility was observed. Flexirubin-type pigments were absent. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain JC2675^T represented a distinct phyletic line that reflected a novel generic status within the family *Flavobacteraceae* with relatively low gene sequence similarities (<95.7 %) to other recognized genera. The predominant isoprenoid quinone (MK-6) and DNA G+C content (30 mol%) were consistent with the assignment of the novel strain to the family *Flavobacteriaceae*, but overall phenotypic traits demonstrated that the novel strain was not closely affiliated with any previously described genus. Based on data from a study using a polyphasic taxonomic approach, it is proposed that strain JC2675^T represents a new genus and novel species belonging to the family *Flavobacteriaceae*, for which the name *Pontirhabdus pectinivorans* gen. nov., sp. nov. is proposed. The type strain of the type species is JC2675^T (=KACC 14153^T=JCM 17107^T).

At the time of writing, the bacterial family *Flavobacteriaceae* encompasses 87 genera with validly published names. Members of this family have diverse ecological niches and dynamic physiological features even within a genus (Bernardet & Nakagawa, 2006). One of the major characteristics of this family is that most of its members are pigmented by carotenoid or flexirubin. The name *Flavobacteriaceae* was derived from the etymology of the type genus of this family, *Flavobacterium*, and means a yellow-coloured bacterium (L. adj. *flavus*, yellow; L. neut. n. bacterium, a small rod; N.L. neut. n. *Flavobacterium*, a yellow bacterium). An additional yellow-pigmented flavobacterial strain, designated JC2675^T, was isolated from seawater and was studied according to the minimal standards for describing new taxa of the family *Flavobacteriaceae* (Bernardet *et al.*, 2002). On the basis of evidence derived from a polyphasic taxonomic approach, the isolate represents a novel genus in the family *Flavobacteriaceae*.

Strain JC2675^T was isolated from a seawater sample from Jeju Island, Korea, using a standard dilution plating method on marine agar 2216 (MA; Difco). The isolate was routinely cultured on MA and maintained as glycerol suspensions (20 %, w/v) at –80 °C. *Algibacter lectus* KCTC 12103^T, *Algibacter mikhailovii* KCTC 12710^T, *Lacinutrix copepodicola* ACAM 1055^T, *Lacinutrix algicola* KCCM 42313^T and *Lacinutrix mariniflava* KCCM 42306^T were selected as reference strains and evaluated together under identical experimental conditions to those for strain JC2675^T.

The 16S rRNA gene was enzymically amplified from a single colony. Primers, PCR conditions and sequencing methods have been described elsewhere (Chun & Goodfellow, 1995). Preliminary sequence identification against the 16S rRNA gene sequences held in the EzTaxon database of prokaryotic type strains with validly published names (Chun *et al.*, 2007) indicated that this isolate belonged to the family *Flavobacteriaceae*. The nearly complete 16S rRNA gene sequence of strain JC2675^T (1410 bp) was aligned manually against representatives of the family *Flavobacteriaceae* using the bacterial 16S rRNA secondary structure model (Woese *et al.*, 1980). The regions available for all sequences (positions 93–182, 213–820 and 850–1437; *Escherichia coli* numbering system) showed unambiguous alignment and were used to construct phylogenetic trees. Alignment and neighbour-joining tree (Saitou & Nei, 1987) analyses were carried out using the JPHYDIT program (Jeon *et al.*, 2005). An

The GenBank accession number for the 16S rRNA gene sequence of strain JC2675^T is HM475134.

Supplementary figures are available with the online version of this paper.

evolutionary distance matrix for the neighbour-joining tree was generated according to the model of Jukes & Cantor (Jukes & Cantor, 1969) and the resultant tree topologies were evaluated by bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. A Bayesian inference was performed with MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) with the General Time Reversible evolutionary model with a gamma-distributed rate variation. The program was run for 30 000 000 generations with a sample frequency of 100 and a burn-in of 100 000. A maximum-likelihood (Felsenstein, 1993) tree was constructed using PAUP 4.0 (Swofford, 1998) with a heuristic search option and 100 replicates. On the basis of 16S rRNA gene sequence analyses, strain JC2675^T showed the highest sequence similarity to *A. lectus* KMM 3902^T (95.6 %), followed by *A. mikhailovii* LMG 23988^T (95.3 %), *Gaetbulibacter saemankumensis* SMK-12^T (94.8 %), *Flaviramulus basaltis* H35^T (94.5 %), *L. copepodicola* DJ3^T (94.4 %), the genus *Olleya* (94.3–94.5 %), the genus *Bizionia* (93.8–94.2 %), *L. algicola* AKS293^T (94.2 %), *L. mariniflava* AKS432^T (94.0 %), *Gaetbulibacter marinus* IMCC 1914^T (93.6 %) and *Formosa algae* KMM 3553^T (93.3 %). No other taxon showed more than 93 % sequence similarity with the new isolate. Phylogenetic analysis revealed that strain JC2675^T could be clearly distinguished from any recognized genus (Fig. 1). Strain JC2675^T formed a distinct phyletic line within the radiation of the family *Flavobacteriaceae* and the branching position varied considerably depending on the tree-making algorithm used and the taxa included in the analyses. In the neighbour-joining tree containing the type strains of type species belonging to the family *Flavobacteriaceae*, the isolate was positioned between the genera *Algibacter* and *Lacinutrix* and appeared to be more related to the genus *Algibacter* than to the genus *Lacinutrix* (Fig. 1). However, when the number of taxa analysed together was enlarged by adding the type strains of additional species, the isolate was positioned far from the genus *Algibacter* and branched as a sister group of the genus *Lacinutrix* (see Supplementary Fig. S1a in IJSEM Online). In the maximum-likelihood (Supplementary Fig. S1b) and Bayesian trees (Supplementary Fig. S1c), the novel strain branched in the middle of the clade containing the genera *Algibacter*, *Hyunsoonleella* and *Jejuia*. Thus, based on the 16S rRNA gene sequence analyses, strain JC2675^T appeared to represent a new genus and a novel species belonging to the family *Flavobacteriaceae*.

Growth on seawater-supplemented bacterial media including cetrimide agar (Difco), MacConkey agar (Difco) and yeast extract-free ZoBell's agar (ZoBell, 1941) was tested. The temperature (5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 °C), pH (between pH 5 and pH 10.5, with increments of 0.5 pH units; adjusted with 10M KOH or HCl) and NaCl or sea salts (Sigma) concentration [0, 0.5, 1, 2, 3, 4, 5, 7, 10, 12, 15 and 20 % (w/v)] ranges for growth were determined using synthetic ZoBell medium. Growth under anaerobic and microaerophilic conditions was assessed using anaerobically prepared MA in an anaerobic chamber (10 % CO_2, 10 % H_2, 80 % N_2; Sheldon Manufacturing) and CampyPak Plus systems (5–15 % O_2 and 5–12 % CO_2; BBL).

Cellular morphology and motility were examined after growth on MA at 30 °C for 2 days by transmission electron and phase-contrast microscopy, respectively (see Supplementary Fig. S2 in IJSEM Online). Gliding motility was observed by direct phase-contrast microscopic examination of cells initially grown in marine broth 2216 (Difco) at 30 °C for 2 days and subsequently incubated for 16, 48, and 72 h on microscope slides coated with MA (0.7 % agar), according to the method described by Bowman (2000). The presence of flexirubin-type pigments was determined by flooding the cell mass taken from agar plates with 20 % (w/v) KOH and confirmed by examining the bathychromatic shift of the absorbance spectrum of ethanol and alkaline-ethanol extracts of lysed cells (Weeks, 1981).

Standard physiological and biochemical tests were performed as described previously (Smibert & Krieg, 1994). The ability of the novel strain to hydrolyse alginic acids (0.5 %, Sigma), casein (5 % skimmed milk, Difco), microcrystalline cellulose (0.5 %, Sigma), chitin (0.5 %, Sigma), egg yolk (5 %, Oxoid), elastin (0.5 %, Sigma), pectin (0.5 %, Sigma), starch (0.2 %, Difco), Tween 20 (1 %, Sigma), Tween 40 (1 %, Junsei), Tween 60 (1 %, Junsei) and Tween 80 (1 %, Sigma) was tested using MA as the basal medium. Decomposition of adenine (0.5 %, Sigma), hypoxanthine (0.5 %, Sigma), L-tyrosine (0.5 %, Sigma) and xanthine (0.4 %, Sigma) was tested using MA according to Gordon *et al.* (1974). Other enzymic activities were determined using the API 20NE, API 20E and API ZYM (bioMérieux) test kits. Acid production from carbohydrates was tested by using the API 50CH kit with CHB/E medium (bioMérieux) supplemented with half-strength seawater (4 % sea salts in distilled water, Sigma). Utilization of carbohydrates as sole carbon source was tested using the API 50CH kit with AUX medium (bioMérieux) supplemented with half-strength seawater. The API kits were inoculated with a heavy bacterial suspension in half-strength artificial seawater and the data were recorded for up to five consecutive days.

The DNA G+C content was determined by the thermal denaturation method (Marmur & Doty, 1962). Menaquinone was isolated from 3-day-old cells according to Minnikin *et al.* (1984) and analysed by HPLC (Waters) as described by Collins (1985). For fatty acid analysis, strain JC2675^T and the five reference strains were grown on MA at 30 °C (strain JC2675^T), 25 °C (*Algibacter* strains) and 15 °C (*Lacinutrix* strains) for 3 days. Extraction of fatty acid methyl esters and separation by GC were performed by using the Instant FAME method of the Microbial Identification System (MIDI) version 6.1 and the RTSBA6 6.10 database. The fatty acids were iso-$C_{15:0}$ 3-OH (16.7 %), iso-$C_{15:1}$ G (14.7 %), iso-$C_{17:0}$ 3-OH (10.7 %), anteiso-$C_{15:0}$ (7.7 %), iso-$C_{15:0}$ (7.6 %), iso-$C_{16:0}$ 3-OH

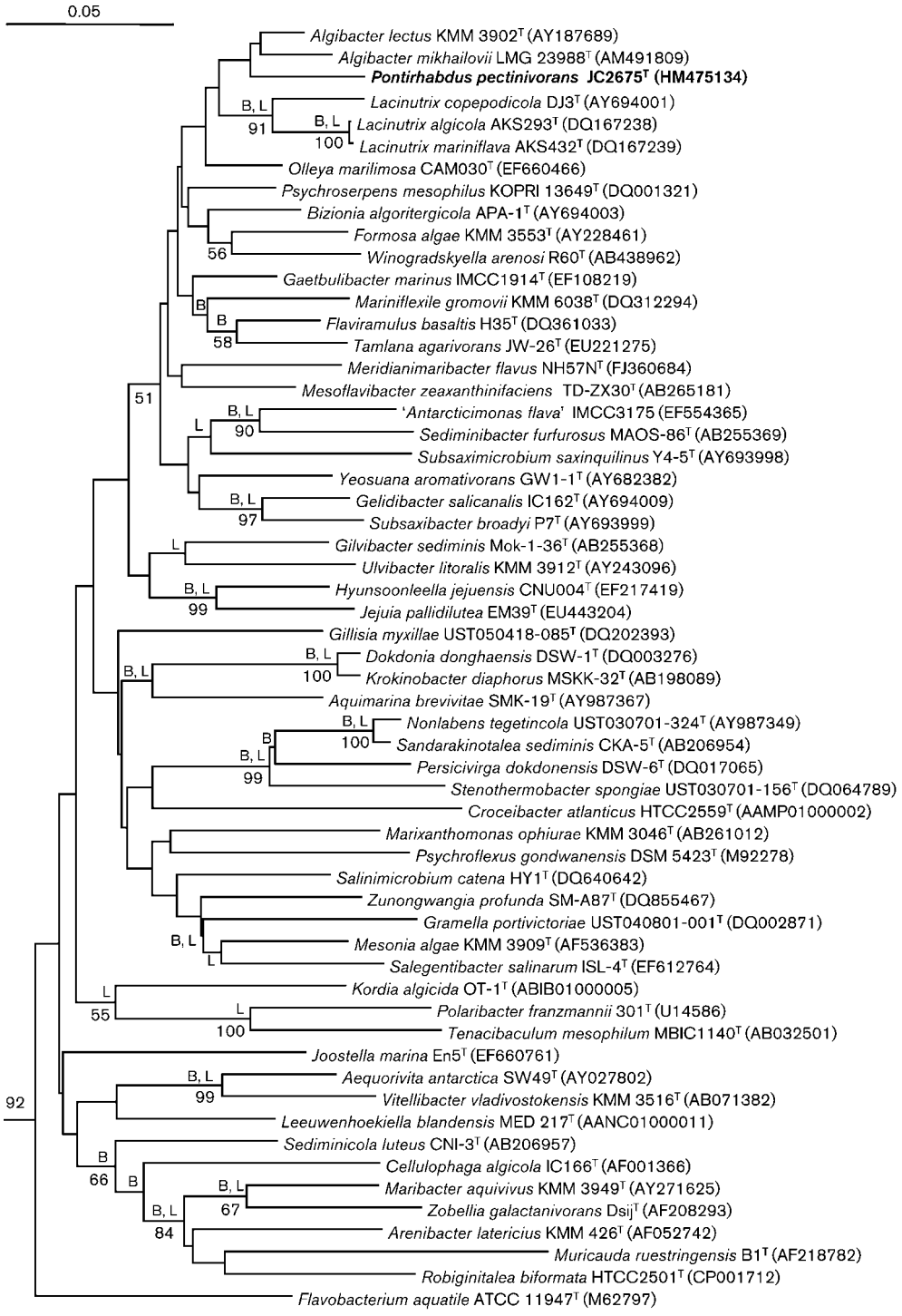

Fig. 1. Neighbour-joining tree based on a representative set of 16S rRNA gene sequences of the family *Flavobacteriaceae*. The numbers at the nodes are percentages of bootstrap support (>50 %) from 1000 resampled datasets. Letters at the nodes indicate that the corresponding nodes (groupings) were recovered also in Bayesian (B) and/or in maximum-likelihood (L) tree-inferring methods. *Helicobacter pylori* NCTC 11637^T (GenBank no. Z25741) was used as an outgroup (not shown). Bar, 0.05 nt substitution per position.

Table 1. Characteristics that differentiate strain JC2675^T from other related genera in the family *Flavobacteriaceae*

Taxa: 1, Strain JC2675^T (data from this study); 2, *Algibacter* (*n*=2; all data from this study except the DNA G+C content); 3, *Lacinutrix* (*n*=3; all data from this study except the DNA G+C content); 4, *Hyunsoonleella jejuensis* CNU004^T (Yoon *et al.*, 2010); 5, *Jejuia pallidilutea* EM39^T (Lee *et al.*, 2009); 6, *Olleya* (*n*=2; Lee *et al.*, 2010; Nichols *et al.*, 2005); 7, *Flaviramulus basaltis* H35^T (Einen & Ovreås, 2006). *n*, Number of type strains in each genus; +, positive; –, negative; W, weakly positive; ND, no data available; V, varies among different strains within the same genus; VA, variable results depending on test methods; tr, trace. All taxa are aerobic and positive for catalase, alkaline phosphatase and hydrolysis of Tween 80; negative for nitrate reduction (not determined for *F. basaltis*). Strain JC2675^T and members of the genera *Algibacter* and *Lacinutrix* are positive for leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities; negative in tests for the decomposition of adenine, hypoxanthine or xanthine, the production of indole and activities of arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, lipase (C14), β-glucuronidase, β-glucosidase, α-mannosidase and α-fucosidase.

Characteristic	1	2	3	4	5	6	7
DNA G+C content (mol%)	30	31–35*	34.7–37*	37.7	34.6	35–42	31.4
Major fatty acids							
ai-$C_{15:0}$	7.7	5.1–7.9	5.4–14.2	4.6	9.3	2.9–4.7	6.2
i-$C_{15:0}$	7.6	8.3–9.2	11.1–16.6	18.4	14.0	16.9–17.2	19.4
i-$C_{15:0}$ 3-OH	16.7	5.6–10.8	3.2–12.6	11.6	8.6	5.6–12.1	13.6
i-$C_{15:1}$	14.7	10.5–15.8	12.3–15.2	17.7	13.9	15.0–15.7	21.2
i-$C_{16:0}$ 3-OH	6.4	0.0–4.5	7.3–10.4	0.0	7.8	tr–3.7	4.5
i-$C_{17:0}$ 3-OH	10.7	11.6–12.0	5.6–13.0	16.6	10.1	9.9–12.0	13.3
Summed feature 3†	3.3	8.2–29.6	5.8–8.0	5.0	5.4	8.9–9.9	4.9
Requirement for sea salts‡	+	V	V	+	ND	–	+
Requirement for yeast extract	–	–	–	ND	ND	+	+
Flexirubin-type pigments	–	–	V§	–	–	–	–
Acid production from carbohydrates	+	+	V	ND	ND	+	+
Acid production from glucose	–	–	–	–	ND	+	ND
Gliding motility	+	V‖	+¶	–	–	+	+
Oxidase	+	+	+	+	+	+	–
Urease	–	–	–	–	–	–	+
Citrate utilization	–	–	V	ND	ND	+	ND
Production of H_2S	–	–	–	–	ND	–	+
Production of acetoin	+	+	+	+	ND	–	ND
Degradation of:							
Agar	–	+	–	–	ND	–	–
Alginic acids	–	+	–	ND	ND	ND	ND
Casein	–	–	+	–	–	+	ND
Cellulose	–	–	–	+	–	–	–
Chitin	–	–	–	ND	ND	ND	–
Egg yolk	clear#	clear	V	ND	ND	ND	ND
Elastin	+	–	V	ND	ND	+	ND
Aesculin	+	+	V	+	+	+	+
Gelatin	+	VA	+	+	–	+	+
Pectin	+	–	–	ND	ND	ND	ND
Starch	–	+	V	–	+	V	+
Tween 20	+	V	+	ND	+	+	+
Tween 40	+	V	+	ND	ND	+	+
Tween 60	+	V	+	ND	ND	+	ND
L-Tyrosine	+	+	+	ND	–	V	+
API ZYM							
Esterase (C4)	–	–	–	+	+	V	ND
Esterase lipase (C8)	W	–	–	+	+	+	ND
Cystine arylamidase	–	–	–	+	+	+	ND
Trypsin	+	–	V	+	–	–	ND
α-Chymotrypsin	–	–	–	–	+	V	ND
α-Galactosidase	–	–	–	+	–	–	ND
β-Galactosidase	–	V	–	+	–	–	ND

Table 1. cont.

Characteristic	1	2	3	4	5	6	7
α-Glucosidase	−	−	−	−	+	−	ND
N-Acetyl-β-glucosaminidase	−	V	−	+	+	−	ND

*Data from (Bowman & Nichols, 2005; Nedashkovskaya *et al.*, 2004, 2007, 2008).
†Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 contained $C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$.
‡Strain ACAM 1055^T cannot grow in the presence of NaCl.
§*L. copepodicola* ACAM 1055^T produced flexirubin-type pigments in this study, in contradiction to a previous report (Bowman & Nichols, 2005).
¶*A. mikhailovii* KCTC 12710^T did not show gliding motility, contrary to data from a previous report (Nedashkovskaya *et al.*, 2007).
‖*Lacinutrix* species were positive for gliding motility, in contradiction to previous reports (Bowman & Nichols, 2005; Nedashkovskaya *et al.*, 2008).
#Egg yolk decomposition was negative but a clear zone was formed around colonies.

(6.4 %), $C_{15:0}$ 3-OH (5.0 %), anteiso-$C_{15:1}$ A (3.6 %), summed feature 3 [comprising $C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$ (3.3 %)], $C_{16:0}$ 3-OH (2.8 %), $C_{16:0}$ (2.6 %), $C_{17:0}$ 2-OH (2.5 %), iso-$C_{13:0}$ (2.2 %), $C_{15:0}$ 2-OH (2.1 %), $C_{15:1}\omega6c$ (2.1 %), anteiso-$C_{13:0}$ (1.6 %), iso-$C_{14:0}$ (1.4 %), $C_{17:0}$ 3-OH (1.1 %) and $C_{13:0}$ (1.1 %). The results of the morphological, cultural, biochemical, physiological and chemotaxonomic tests are presented in the species description.

Phylogenetic analyses based on the 16S rRNA gene sequences showed that JC2675^T represented a distinct phyletic line that reflected a novel genus status. Overall phenotypic traits also demonstrated that the novel strain was not closely affiliated with any previously described genera (Table 1). Thus, based on data derived using a polyphasic taxonomic approach, it is proposed that strain JC2675^T represents a new genus and a novel species belonging to the family *Flavobacteriaceae*, for which the name *Pontirhabdus pectinivorans* gen. nov., sp. nov. is proposed.

Description of *Pontirhabdus* gen. nov.

Pontirhabdus (Pon.ti.rhab′dus. L. n. *pontus* the sea; N.L. fem. n. *rhabdus* a rod, wand; N.L. fem. n. *Pontirhabdus* a rod that grows in the sea).

Gram-reaction-negative, oxidase- and catalase-positive and aerobic. Cells are rods with rounded ends and are able to glide. Spores are not formed. Produces non-diffusible carotenoid pigments, but flexirubin-type pigments are absent. Requires sea salts, but not yeast extract, for growth. Major isoprenoid quinone is MK-6. Predominant cellular fatty acids are iso-$C_{15:0}$ 3-OH, iso-$C_{15:1}$ G, iso-$C_{17:0}$ 3-OH, anteiso-$C_{15:0}$ and iso-$C_{15:0}$. DNA G+C content is 30 mol%. The maximum absorption peak of pigments is at 452 nm and the next shoulder peak is at 480 nm. A member of the family *Flavobacteriaceae*, class *Flavobacteria*, phylum '*Bacteroidetes*'. The type species is *Pontirhabdus pectinivorans*.

Description of *Pontirhabdus pectinivorans* sp. nov.

Pontirhabdus pectinivorans (pec.ti.ni.vo′rans. N.L. n. *pectinum* pectin; L. part. adj. *vorans* devouring; N.L. part. adj. *pectinivorans* pectin-devouring).

Displays the following characteristics in addition to those given for the genus. Grows under aerobic and microaerobic conditions, but not under anaerobic conditions. Cells are approximately 0.4–0.6 × 1.0–2.4 μm. Colonies are yellow, convex, translucent, glistening, circular with entire margins. Glides on agar plates. Does not grow on cetrimide or MacConkey agar. Growth occurs at pH 5.5–8.5 (optimum, pH 7) and 4–35 °C (optimum, 30 °C). Requires sea salts concentration of 1–7 % (w/v) (optimum, 2–3 %) for growth and cannot grow on sea salts-free ZoBell's medium supplemented with NaCl only. Produces acetoin. Does not reduce nitrate to nitrite or nitrogen. Does not produce indole or H_2S. Does not utilize citrate. Acid is not produced from glucose. Decomposes elastin, aesculin, gelatin, pectin, Tweens 20, 40, 60 and 80 and L-tyrosine, but not agar, adenine, alginic acids, casein, cellulose, chitin, egg yolk, hypoxanthine, starch or xanthine. Positive in tests for alkaline phosphatase, leucine arylamidase, valine arylamidase, trypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase, and weakly positive for esterase lipase (C8), but negative for urease, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, esterase (C4), lipase (C14), cystine arylamidase, α-chymotrypsin, α-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. β-Galactosidase activity is negative in API ZYM and API 20E kits, but positive in the API 20NE kit. On the basis of the API 50CH system, utilizes the following carbohydrates as sole carbon source: D-xylose, D-galactose, D-mannose, salicin, cellobiose, maltose, lactose (bovine origin) and sucrose, but cannot utilize D-arabinose, D-glucose, D-fructose, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, *N*-acetylglucosamine, amygdalin, arbutin, aesculin ferric citrate, melibiose, trehalose, raffinose, starch, gentiobiose, methyl β-D-xylopyranoside, glycogen,

turanose, glycerol, erythritol, L-arabinose, D-ribose, L-xylose, D-adonitol, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, inulin, melezitose, xylitol, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate or potassium 5-ketogluconate. On the basis of the API 50CH system, is able to produce acid from D-xylose, D-galactose, D-glucose, D-mannose, L-rhamnose, amygdalin, aesculin ferric citrate, cellobiose, maltose, lactose (bovine origin), sucrose and gentiobiose. Produces acid weakly from D-fructose and salicin but no production from D-arabinose, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, *N*-acetylglucosamine, arbutin, melibiose, trehalose, raffinose, starch, methyl β-D-xylopyranoside, glycogen, turanose, glycerol, erythritol, L-arabinose, D-ribose, L-xylose, D-adonitol, L-sorbose, dulcitol, inositol, D-mannitol, D-sorbitol, inulin, melezitose, xylitol, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate or potassium 5-ketogluconate. The fatty acids include iso-$C_{15:0}$ 3-OH, iso-$C_{15:1}$ G, iso-$C_{17:0}$ 3-OH, anteiso-$C_{15:0}$, iso-$C_{15:0}$, iso-$C_{16:0}$ 3-OH, $C_{15:0}$ 3-OH, anteiso-$C_{15:1}$ A, summed feature 3 (comprising $C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$), $C_{16:0}$ 3-OH, $C_{16:0}$, $C_{17:0}$ 2-OH, iso-$C_{13:0}$, $C_{15:0}$ 2-OH, $C_{15:1}\omega6c$, anteiso-$C_{13:0}$, iso-$C_{14:0}$, $C_{17:0}$ 3-OH and $C_{13:0}$.

The type strain, JC2675^T (=KACC 14153^T=JCM 17107^T), was isolated from a seawater sample from Jeju Island, Korea. The DNA G+C content of the type strain is 30 mol%.

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