Gilvimarinus japonicus sp. nov., a cellulolytic and agarolytic marine bacterium isolated from coastal debris

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A cellulolytic and agarolytic bacterial strain, designated 12-2^T, was isolated from a piece of cotton rope fragment washed ashore on a beach and was studied phenotypically, genotypically and phylogenetically. Analyses of 16S rRNA and *gyrB* gene sequences and DNA base composition suggested that the strain is a member of the genus *Gilvimarinus*. However, levels of 16S rRNA and *gyrB* gene sequence similarity between it and the type strains of *Gilvimarinus* species were no higher than 97.9 and 78.7 %, respectively, suggesting that the strain is distinct. Moreover, the results of DNA–DNA hybridization experiments and physiological characterization clearly differentiated the strain from its closest neighbours. The strain is therefore considered to represent a novel species of the genus *Gilvimarinus*, for which the name *Gilvimarinus japonicus* sp. nov. is proposed. The type strain is 12-2^T (=NBRC 111987^T=KCTC 52141^T).

A number of cellulolytic microbes have been isolated from terrestrial environments and are known to contribute to cellulose biodegradation in soil (Bhat & Bhat, 1997), but knowledge of cellulolytic microbes in the marine environment remains limited. Therefore, it is likely that many cellulolytic marine strains remain to be characterized, and it is necessary to isolate them to elucidate their role in cellulose biodegradation in the ocean. We recently isolated a cellulolytic bacterial strain, designated 12-2^T, from a piece of cotton rope washed ashore on a beach in Yamaguchi, Japan. Analyses of the 16S rRNA gene partial sequence and the chemotaxonomic characteristics of the strain indicated that it belonged to the genus *Gilvimarinus*. The genus *Gilvimarinus* was proposed by Du *et al.* (2009) as a novel genus in the class *Gammaproteobacteria*. Three members, *Gilvimarinus chinensis*, '*Gilvimarinus agarilyticus*' and *Gilvimarinus polysaccharolyticus*, have been reported thus far. Interestingly, all species were isolated from the Yellow Sea, a marginal sea of the Pacific Ocean, and possess agar-hydrolysing activity, implying their role in seaweed decomposition in the marine ecosystem (Du *et al.*, 2009; Kim *et al.*, 2011; Cheng *et al.*, 2015). Furthermore, analysis of 16S rRNA gene sequences suggested that strain 12-2^T is distinct from known *Gilvimarinus* species. Therefore, analyses of DNA gyrase subunit B (*gyrB*) gene sequences, DNA base composition, phenotypic and biochemical traits and DNA–DNA hybridization were performed to precisely identify the strain. Based on our results, strain 12-2^T should be classified as representing a novel species of the genus *Gilvimarinus*.

A piece of cotton rope fragment (approximately 15 cm in length and 2 cm in diameter) found on a beach in Yamaguchi, Japan (34° 24′ N 131° 23′ E), was collected in a sterilized polyethylene bag and brought back to our laboratory. The rope fragment was then unravelled using an autoclaved pair of tweezers on a clean bench, suspended in 40 ml autoclaved artificial seawater (Marine Art SF-1; Tomita Pharmaceuticals) and vortexed vigorously for 1 min. The supernatant (1 ml) was added to 20 ml of autoclaved artificial seawater supplemented with 1.0 g of Whatman no. 7 filter paper, which was cut into fragments (3×20

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA and *gyrB* gene sequences of strain 12-2^T are LC125208 and LC125209, respectively.

Five supplementary figures are available with the online Supplementary Material.

mm average size) by a paper shredder, as a sole carbon source and cultured at 23 °C at 120 r.p.m. We used a temperature of 23 °C for the enrichment, because ambient temperature at the sampling location was 23 °C. Microbial growth was detected by 2 weeks and 20 µl of the culture was inoculated and spread on marine agar 2216 (Becton Dickinson) by a bacterial spreader to isolate strain 12-2^T.

Genomic DNA was purified using the procedures described by Pospiech & Neumann (1995). Bacterial cells were grown in 100 ml marine broth 2216 (Becton Dickinson) for 2 weeks at 23 °C and harvested by centrifugation (3000 ***g*** for 10 min). The harvested bacterial cells were suspended in Tris−EDTA buffer (pH 8.0) and lysed with lysozyme (final concentration, 2 mg ml^{-1}) and SDS (final concentration, 0.5 %). DNA was then recovered from the lysate by phenol−chloroform extraction, followed by RNase treatment, CTAB treatment and ethanol precipitation. Each procedure was performed twice to obtain highly pure DNA. The partial 16S rRNA (1422 bases) and *gyrB* (1127 bases) gene sequences were amplified by PCR using KOD FX Neo DNA polymerase (Toyobo). The universal primer set corresponding to positions 8–27 (forward primer) and 1492–1510 (reverse primer) of the *Escherichia coli* numbering system (Weisburg *et al.*, 1991) was used for 16S rRNA gene amplification, and the following primer pair was used for *gyrB* gene amplification: UP1 (forward: 5′-GAAGTCATCATGACCGTTCTGCAYGC NGGNGGNAARTTYGA-3′) and UP2r (reverse: 5′-AG-CAGGGTACGGATGTGCGAGCCRTCNACRTCNGCRTC NGTCAT-3′) (Yamamoto & Harayama, 1995). The thermal profile involved 45 cycles: 98 °C for 10 s, 54 °C for 30 s and 68 °C for 60 s. The DNA sequences of the strain were compared with all known sequence data in the GenBank, EMBL and DDBJ databases using the BLAST program and the Ribosomal Database Project (http://rdp.cme.msu.edu/). Multiple alignment and reconstruction of phylogenetic trees by the maximum-likelihood method were performed using the MEGA 6 computer program (Edgar, 2004; Tamura *et al.*, 2013). Robustness of the topology of the phylogenetic trees was evaluated by bootstrap analysis with 1000 replications. A phylogenetic tree of the strain and its related species based on 16S rRNA gene sequences is shown in Fig. 1(a). The 16S rRNA gene sequence of the strain was positioned within the genus *Gilvimarinus*, which contains only three strains assigned to the species level (‘*G. agarilyticus*’ KCTC 23325^T, *G. polysaccharolyticus* JCM 19198^T and *G. chinensis* DSM 19667^T). Levels of 16S rRNA gene sequence similarity between strain 12-2^T and known species were 97.9 % (‘*G. agarilyticus*’ KCTC 23325^T), 96.9 % (*G. polysaccharolyticus* JCM 19198^T) and 94.7 % (*G. chinensis* DSM 19667^T). These values are sufficiently low to suggest that the strain is not conspecific to the closest species (Keswani & Whitman, 2001; Stackebrandt & Ebers, 2006). A phylogenetic tree based on *gyrB* gene sequences was also reconstructed (Fig. 1b), because this gene is reported to be useful to define the phylogenetic relationships among *Gammaproteobacteria* (Hatano *et al.*, 2003). The *gyrB* gene sequence similarities between strain 12-2^T and known species were 78.7 % (‘*G. agarilyticus*’ KCTC 23325^T), 76.8 % (*G. polysaccharolyticus* JCM 19198^T) and 67.9 % (*G. chinensis* DSM 19667^T). Hatano *et al.* (2003) reported that a *gyrB* gene sequence similarity of approximately 96.5 % would correspond to 70 % DNA–DNA relatedness, thereby suggesting that strain 12-2^T is different from the closest related species.

DNA base composition, i.e. guanine-plus-cytosine (G+C) content, was determined by HPLC, as described by Tamaoka & Komagata (1984). The G+C content for the genomic DNA of strain 12-2^T was 52.3 mol%, which is consistent with values (48−53 mol%) reported for other members of the genus *Gilvimarinus* (Cheng *et al.*, 2015). Total lipids were extracted from bacterial cells according to the method described by Bligh & Dyer (1959), and phospholipids were analysed using two-dimensional TLC. Crude lipids of strain 12-2^T were separated on silica gel 60 TLC plates (Merck) using two solvent systems, one being chloroform/methanol/water (65 : 25 : 4, by vol.) and the other chloroform/methanol/acetic acid/water (80 : 12 : 15 : 4, by vol.). Crude lipids of ‘*G. agarilyticus*’ KCTC 23325^T obtained from the Korean Collection for Type Cultures (KCTC) were used as a control. A 50 % sulphuric acid solution was used to detect the lipid spots. Zinzadze (Dittmer) reagent and ninhydrin were used to detect phosphate and free amino groups, respectively. The lipids from strain 12-2^T contained diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine (Fig. S1, available in the online Supplementary Material), which are markers for the genus *Gilvimarinus* (Cheng *et al.*, 2015). Fatty acid methyl esters were prepared using a Nakarai fatty acid methylation kit and were analysed using a Parvum2 gas chromatograph-mass spectrometer (GC/MS; Shimadzu) under the following conditions: column, Rxi-5ms capillary column (30 m×0.25 mm i.d.; Restek); injection volume, 1 µl; carrier gas, helium (17 ml min^{-1}); temperature gradient, 70–330 °C at 5 °C min^{-1}, and 330 °C for 3 min. The energy for electron impact ionization was 70 eV. ‘*Gilvimarinus agarilyticus*’ KCTC 23325^T and *G. polysaccharolyticus* JCM 19198^T, which was obtained from the Japan Collection of Microorganisms (JCM), were used as controls. Table 1 shows the fatty acid compositions of the tested strains. Of the characteristic fatty acids of the genus *Gilvimarinus* ($C_{16:0}$, $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH, $C_{18:1}\omega7c$; Cheng *et al.*, 2015), $C_{16:0}$ and $C_{16:1}\omega7c$ were found in strain 12-2^T as well as its closest neighbours. Interestingly, $C_{18:1}\omega7c$ was not found in the fatty acid profile of strain 12-2^T, although it is one of the major fatty acids in the genus *Gilvimarinus*. iso-$C_{15:0}$ was found in all tested strains, but we could not isolate or detect its hydroxy derivative (iso-$C_{15:0}$ 2-OH) due to a limitation in our GC/MS analytical conditions.

The isoprenoid quinone type was determined by one-dimensional TLC and HPLC, according to the methods described by Yamada & Kuraishi (1982). Total acetone-soluble cell extracts were separated by one-dimensional TLC with benzene as the eluent. Isoprenoid length was analysed by HPLC using an Inertsil ODS-3V column (GL Science) for monitoring of UV absorption at 275 nm. As the mobile phase (flow rate, 0.7 ml min^{-1}), a mixture of 70 % methanol/30 % 2-propanol was used. ‘*Gilvimarinus agarilyticus*’ KCTC 23325^T

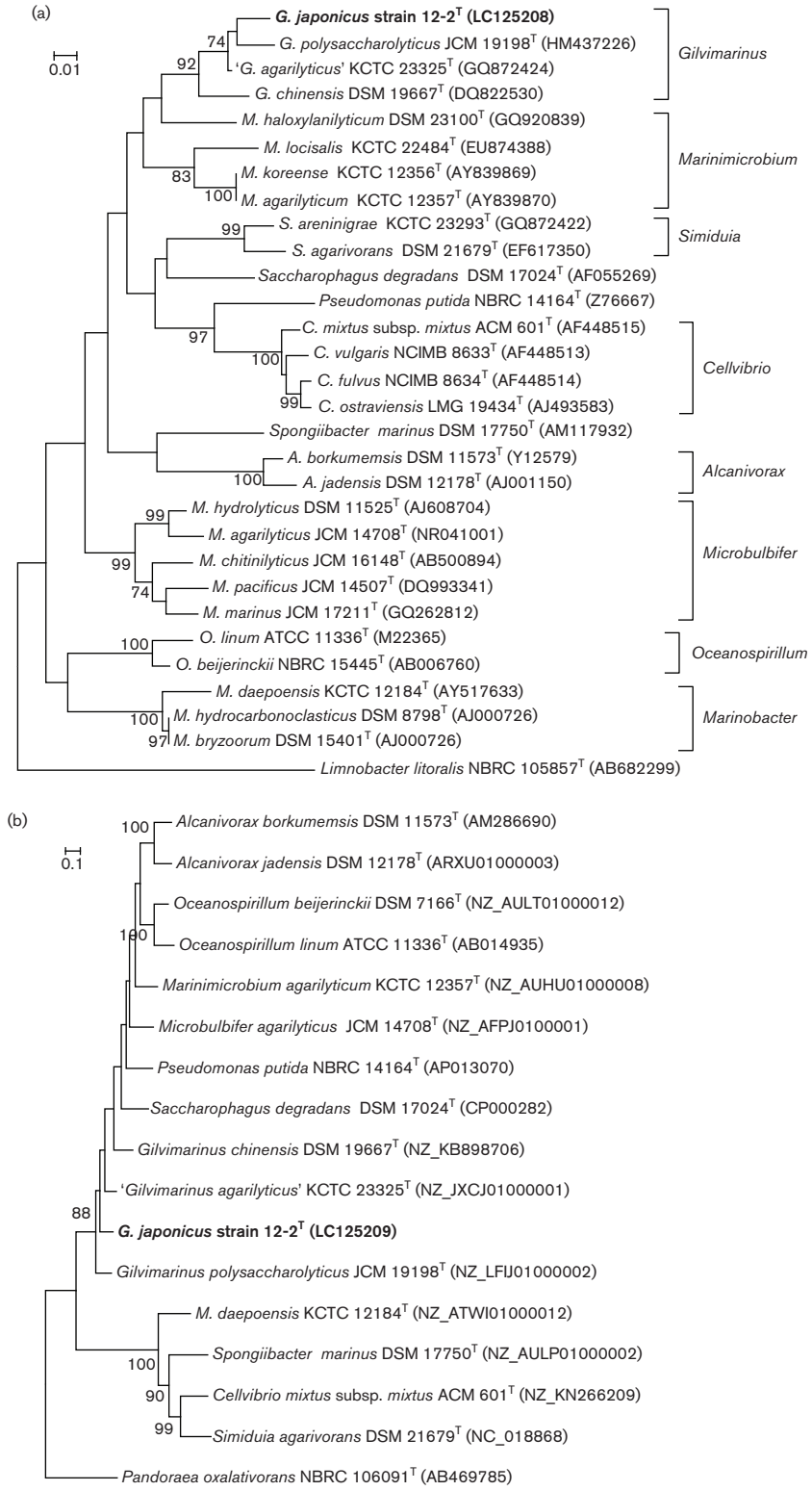

Fig. 1. Phylogenetic tree reconstructed by the maximum-likelihood method based on 16S rRNA gene (a) and *gyrB* gene (b) sequences of strain 12-2^T and related species. The 16S rRNA gene sequence of *Limnobacter litoralis* NBRC 105857^T (AB682299) and *gyrB* gene sequence of *Pandoraea oxalativorans* NBRC 106091^T (AB469785) were used as the outgroup. Bootstrap values $\geq$ 70 % (of 1000 samplings) are shown at the internodes. Bars, evolutionary distance (K_{nuc}) of 0.01 and 0.1 for the 16S rRNA gene and *gyrB* gene, respectively (Kimura, 1980).

Table 1. Fatty acid compositions of strain 12-2^T and its closest neighbouring species

Strains: 1, 12-2^T; 2, '*Gilvimarinus agarilyticus*' KCTC 23325^T; 3, *G. polysaccharolyticus* JCM 19198^T. Values are percentages of total fatty acids. ND, Not detected (<0.1 %).

Fatty acid	1	2	3
Saturated			
$C_{14:0}$	ND	3.3	1.9
$C_{15:0}$	3.6	2.6	2.6
$C_{16:0}$	24.9	21.5	17.2
$C_{17:0}$	4.1	5.0	2.4
$C_{18:0}$	9.3	10.5	2.3
Unsaturated			
$C_{16:1}\omega 9c$	14.7	15.3	34.4
$C_{16:1}\omega 7c$	5.8	5.0	0.2
$C_{18:1}\omega 9c$	5.1	2.4	ND
$C_{18:1}\omega 7c$	ND	21.1	18.2
$C_{18:1}\omega 6c$	4.0	1.1	0.1
Branched chain			
iso-$C_{15:0}$	5.8	2.5	4.6
anteiso-$C_{15:0}$	10.9	3.5	ND
iso-$C_{16:0}$	ND	ND	1.7
anteiso-$C_{16:0}$	ND	ND	ND
iso-$C_{17:0}$	4.8	2.6	0.9
anteiso-$C_{17:0}$	5.4	2.3	6.6
Others (unidentified)	1.6	1.3	6.9

and *G. polysaccharolyticus* JCM 19198^T were used as reference strains. The predominant respiratory quinone of strain 12-2^T was ubiquinone-8 (Q-8), which was also found in '*G. agarilyticus*' KCTC 23325^T and *G. polysaccharolyticus* JCM 19198^T. The presence of Q-8 as the predominant isoprenoid quinone is typical for *Gilvimarinus* species (Cheng *et al.*, 2015).

The phenotypic characteristics of strain 12-2^T were subsequently studied. The Gram reaction was tested as described by Yokota (1999). *Streptomyces abietis* NBRC 109094^T obtained from NITE Biological Resource Center (NBRC) and *E. coli* JM109 (Takara Bio) were used as reference strains. Motility was tested in a semi-solid marine agar containing 0.4 % (w/v) agar. Cell morphology was examined by optical microscopy, scanning electron microscopy and transmission electron microscopy. For scanning electron microscopy, cells of strain 12-2^T grown on marine agar at 30 °C for 7 days were fixed with 2.5 % glutaraldehyde in 50 mM phosphate buffer (pH 7.0) for 4 h at room temperature. The fixed cells were subsequently washed with 1 ml of 50 mM phosphate buffer (pH 7.0) three times, dehydrated with graded (50, 70, 80, 90 and 100 %) ethanol and frozen in 100 % *t*-butanol at −80 °C. The cells were then freeze-dried for 3 h using an FD-1000 freeze drier (Tokyo Rika) and sputter-coated with gold using an SC-704 coater (Sanyu Electron). The coated cells were observed using a JSM-6360 scanning electron microscope (JEOL). For transmission electron microscopy, cells were fixed on a small coverslip (8×6 mm) with 20 mM HEPES buffer (pH 7.4) containing 500 mM NaCl, 1.25 % glutaraldehyde and 2 mg tannic acid ml^{-1} for 20 min at room temperature. After three washes with distilled water, cells on the coverslip were dehydrated with graded (10, 20, 40, 60, 80, 90 and 100 %) ethanol and frozen in 100 % *t*-butanol at −20 °C for 20 min. The cells were then freeze-dried overnight in a plastic desiccator connected to a G-10DA rotary pump (ULVAC), shadowed with platinum from an angle of 22.5° and subsequently coated with carbon in an SVC-700TM/7PS80 coater (Sanyu Electron) to obtain the replica. After hydrofluoric acid treatment to remove the coverslip, the replica was observed using a EM-2100 transmission electron microscope (JEOL), and images were recorded with a KeenView CCD camera (OLYMPUS). Anaerobic growth was assayed by cultivating the strains on marine agar for 1 week under anaerobic conditions with an AnaeroPack Kenki (Mitsubishi Gas Chemical). Oxidase activity was assayed using Bactident oxidase strips (Merck) and catalase activity was determined by bubble production in 3 % hydrogen peroxide solution. The temperature range for growth and optimal growth temperature were determined by cultivation at 4, 10, 15, 20, 25, 28, 30, 32, 35, 37 and 40 °C on marine agar and in 0.1 % (w/v) cellobiose-supplemented marine broth 2216 (CMB), respectively. NaCl tolerance and the optimal NaCl concentration were studied using NaCl-free marine agar and NaCl-free CMB, which were prepared according to the Difco manual (Becton Dickinson), with different NaCl concentrations (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 and 15 %, w/v). The pH range for growth and the optimal pH were determined by cultivating the strain with CMB which was adjusted to pH 5.0–10.0 (in 0.5 pH unit intervals) using appropriate buffers (50 mM MES-NaOH for pH 5.0–6.5, 50 mM HEPES-NaOH for pH 7.0–8.0, 50 mM Tricine-HCl for pH 8.5–9.0 and 50 mM CAPSO-NaOH for pH 9.5–10.0).

The physiological characteristics were examined using API 20NE and API 50CH test strips (bioMérieux) according to the manufacturer's instructions, except that cells were suspended in Marine Art SF-1 artificial seawater for API 20NE and in marine oxidation-fermentation medium (Leifson, 1963) for API 50CH. Results for API tests were observed over a period of 5 days at 30 °C. Susceptibility to antibiotics was determined on marine agar using discs containing the following antibiotics (μg per disc unless stated otherwise): amikacin (30), amoxicillin (10), ampicillin (10), carbenicillin (100), chloramphenicol (30), ciprofloxacin hydrochloride (5), erythromycin (15), gentamicin (10), kanamycin (30), lincomycin (15), nalidixic acid (30), neomycin (30), norfloxacin (30), novobiocin (10), penicillin G (10 IU), polymyxin B (300 U), streptomycin (10), sulfamethoxazole (1.25), tetracycline (30), trimethoprim (5) and vancomycin (30), according to Cheng *et al.* (2015). The inhibition of cell growth by the antibiotics was observed after 5 days of incubation at 30 °C. '*Gilvimarinus agarilyticus*' KCTC 23325^T and *G. polysaccharolyticus* JCM 19198^T were used for comparison.

The optical micrograph of Gram-stained cells revealed that strain 12-2^T was Gram-stain-negative (Fig. S2) and its cells

Table 2. Differential characteristics between strain 12-2^T and its closest phylogenetic neighbours

Strains: 1, 12-2^T; 2, '*G. agarilyticus*' KCTC 23325^T; 3, *G. polysaccharolyticus* JCM 19198^T, 4, *G. chinensis* DSM 19667^T. +, Positive; −, negative. Data for strain 12-2^T, '*G. agarilyticus*' KCTC 23325^T and *G. polysaccharolyticus* JCM 19198^T were obtained from this study, while data for *G. chinensis* DSM 19667^T were taken from Du *et al.* (2009). All strains tested in this study (12-2^T, '*G. agarilyticus*' KCTC 23325^T and *G. polysaccharolyticus* JCM 19198^T) showed positive results for growth at 10, 15, 20, 25 and 30 °C, pH 6−9 and 0–9 % (w/v) NaCl, but negative for growth at 4 and 40 °C, pH 5 and pH 10 and 15 % (w/v) NaCl. All tested strains were positive for oxidase activity, catalase activity, aesculin hydrolysis, *p*-nitrophenyl-β-D-galactopyranoside hydrolysis and mannose assimilation, but negative for assimilation of mannitol, gluconate, capric acid, adipic acid, malic acid, citric acid and phenylacetic acid, indole production, glucose fermentation, gelatinase activity and arginine dihydrolase activity. All were positive for acid production from D-xylose, methyl β-D-xylopyranoside, D-galactose, D-glucose, *N*-acetyl-D-glucosamine, arbutin, aesculin ferric citrate, salicin, cellobiose, maltose, starch, glycogen, gentiobiose and 2-ketogluconic acid, but negative for acid production from glycerol, erythritol, D-arabinose, D-ribose, L-xylose, D-adonitol, L-sorbose, L-rhamnose, dulcitol, inositol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, sucrose, trehalose, inulin, melezitose, raffinose, xylitol, turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconic acid and 5-ketogluconic acid. All tested strains were positive (sensitive) to amoxicillin, ampicillin, chloramphenicol, ciprofloxacin, erythromycin, gentamycin, kanamycin, nalidixic acid, neomycin, norfloxacin, novobicin, polymixin, streptomycin, sulfamethoxazole and tetracycline, but negative (resistant) to penicillin G.

Characteristic	1	2	3	4
Growth at/with:				
4 °C	−	−	+	+
37 °C	−	+	−	+
10 % (w/v) NaCl	+	+	−	+
12 % (w/v) NaCl	+	+	−	−
Activity of (API 20NE):				
Nitrate reduction	−	−	+	−
Urease	+	+	−	+
Glucose assimilation	−	+	−	+
Arabinose assimilation	−	+	−	−
N-Acetyl glucosamine assimilation	+	+	−	−
Maltose assimilation	−	−	+	+
Acid production from (API 50CH):				
L-Arabinose	+	+	−	−
D-Fructose	−	−	+	−
D-Mannose	−	−	+	−
D-Mannitol	−	−	+	+
Amygdalin	+	−	+	+
D-Lactose	−	+	+	+
Melibiose	+	+	−	−
Antibiotic sensitivity				
Amikacin	−	+	+	+
Carbenicillin	−	+	+	+
Lincomycin	−	−	+	+
Trimethoprim	−	−	+	+
Vancomycin	−	−	+	+

were rod-shaped (0.3–0.5 μm in width and 1.5–2.0 μm in length, Fig. S3a). Cultivation of the strain on semi-solid agar suggested that cells were motile. The existence of a flagellum was confirmed by transmission electron microscopy (Fig. S3b). The physiological features that differentiated strain 12-2^T from known *Gilvimarinus* species are presented in Table 2. While all tested strains possessed many common features, several characteristics that discriminate strain 12-2^T from known neighbours were found. For instance, *G. polysaccharolyticus* JCM 19198^T and *G. chinensis* DSM 19667^T can grow at 4 °C, but strain 12-2^T and '*G. agarilyticus*' KCTC 23325^T cannot. In contrast, '*G. agarilyticus*' KCTC 23325^T and *G. chinensis* DSM 19667^T can grow at 37 °C, but strain 12-2^T and *G. polysaccharolyticus* JCM 19198^T cannot. The optimal growth temperature for strain 12-2^T was 28–30 °C (Fig. S4a), which is consistent with that for '*G. agarilyticus*' KCTC 23325^T (25–30 °C), *G. polysaccharolyticus* JCM 19198^T (28–30 °C) and *G. chinensis* DSM 19667^T (25–30 °C) (Du *et al.*, 2009; Kim *et al.*, 2011; Cheng *et al.*, 2015). Strain 12-2^T and '*G. agarilyticus*' KCTC 23325^T can grow in the presence of 0–12 % (w/v) NaCl, but *G. polysaccharolyticus* JCM 19198^T and *G. chinensis* DSM 19667^T cannot grow at 12 % NaCl. The optimal NaCl concentration for strain 12-2^T was 2–3 % (w/v) (Fig. S4b), while that for '*G. agarilyticus*' KCTC 23325^T, *G. polysaccharolyticus* JCM 19198^T and *G. chinensis* DSM 19667^T was 2–3 % (Kim *et al.*, 2011), 1–3 % (Cheng *et al.*, 2015) and 5 % (Du *et al.*, 2009), respectively. The optimal pH for growth of strain 12-2^T was 7.5–8.0 (Fig. S4c), while that for '*G. agarilyticus*' KCTC 23325^T and *G. polysaccharolyticus* JCM 19198^T was 7.0 (Kim *et al.*, 2011; Cheng *et al.*, 2015). Moreover, strain 12-2^T could be distinguished from known *Gilvimarinus* species by resistance to amikacin and carbenicillin, and inability to produce acid from D-lactose.

These results suggest that strain 12-2^T belongs to the genus *Gilvimarinus* but is distinctive from known species. Therefore, to obtain more conclusive information on the relationship between the new strain and other *Gilvimarinus* species, DNA–DNA hybridization experiments were performed using the microplate hybridization method (Ezaki *et al.*, 1989) and photobiotin labelling. Table 3 shows the DNA–DNA hybridization values among these species. Relatively low values (approximately 23 %) were detected among the strain and its known neighbours. A phylogenetically defined species has been proposed to comprise strains that exhibit ≥70 % DNA–DNA hybridization values (Wayne *et al.*, 1987); therefore, our findings clearly indicate that strain 12-2^T is not conspecific to known *Gilvimarinus* species.

The 16S rRNA gene partial sequences of some *Gilvimarinus* strains [*Gilvimarinus* sp. CPA-136, KT324940 (1069 bp); *Gilvimarinus* sp. CPA-143, GenBank accession no. KT324947 (975 bp); and *Gilvimarinus* sp. NSP500, FR750935 (1397 bp)] were recently deposited in the international DNA databases, including GenBank, EMBL and DDBJ. Phylogenetic trees of strain 12-2^T and the above strains based on 16S rRNA gene partial sequences were thus reconstructed (Fig. S5). All strains revealed lineages different from that for strain 12-2^T, and 16S

Table 3. DNA–DNA hybridization values among strain 12-2^{T}and the type strains of closely related *Gilvimarinus* species

Strain	Percentage hybridization with labelled DNA from:		
	12-2^T	*'G. agarilyticus'* 23325^T	*G. polysaccharolyticus* JCM 19198^T
12-2^T	100 (*n*=3)	17.3±0.6 (*n*=3)	23.3±2.3 (*n*=3)
'G. agarilyticus' KCTC 23325^T	17±2.0 (*n*=3)	100 (*n*=3)	27.0±4.0 (*n*=3)
G. polysaccharolyticus JCM 19198^T	4.9±0.5 (*n*=3)	10.0±1 (*n*=3)	100 (*n*=3)

rRNA gene sequence similarities of strains CPA-136, CPA-143 and NSP500 to strain 12-2^T were 98.9, 98.9 and 98.7 %, respectively.

In conclusion, a phylogenetic analysis based on 16S rRNA and *gyrB* gene sequences suggests that strain 12-2^T is distinct from its known neighbours, '*G. agarilyticus*', *G. polysaccharolyticus* and *G. chinensis*. The biochemical and phenotypic differences also support this conclusion. Low DNA−DNA relatedness clearly demonstrated that strain 12-2^T is distinguished from *Gilvimarinus* species with validly published names and is considered to represent a novel species of the genus *Gilvimarinus*, for which the name *Gilvimarinus japonicus* sp. nov. is proposed.

Description of *Gilvimarinus japonicus* sp. nov.

Gilvimarinus japonicus (ja.po′ni.cus. N.L. masc. adj. *japonicus* Japanese, pertaining to the Sea of Japan, where the type strain was isolated).

Cells are rod-shaped (0.3–0.5 μm in width and 1.5–2.0 μm in length) and motile. Colonies are circular and entire, and are dark ivory on marine agar 2216. Cells are motile with a single polar flagellum. Growth occurs at 10–35 °C (optimum 28–30 °C) and at pH 6.0–9.5 (optimum pH 7.5–8.0). Growth occurs in the presence of 0–12 % (w/v) NaCl (optimum 2–3 %). Catalase- and oxidase-positive. In API 20NE tests, cells are positive for urease activity, aesculin hydrolysis, *p*-nitrophenyl-β-D-galactopyranoside hydrolysis and *N*-acetylglucosamine assimilation, but negative for nitrate reduction, arginine dihydrolase activity, gelatin hydrolysis, indole production, D-glucose fermentation, and assimilation of arabinose, mannitol, gluconate, capric acid, adipic acid, malic acid, citric acid and phenylacetic acid. In API 50CH assays, acid is produced from L-arabinose, amygdalin, melibiose, D-xylose, methyl β-D-xylopyranoside, D-galactose, D-glucose, *N*-acetyl-D-glucosamine, arbutin, aesculin ferric citrate, salicin, cellobiose, maltose, starch, glycogen, gentiobiose and 2-ketogluconic acid. Cells contain diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine, and the major cellular fatty acids (>10 %) include $C_{16:1}\omega9c$, anteiso-$C_{15:0}$ and $C_{16:0}$. The major isoprenoid quinone is Q-8.

The type strain, 12-2^T (=NBRC 111987^T=KCTC 52141^T), was isolated from a piece of cotton rope fragment washed ashore on a beach in Yamaguchi, Japan. The DNA G+C content of the type strain is 52.3 mol%.

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