

# *Gilvimarinus polysaccharolyticus* sp. nov., an agar-digesting bacterium isolated from seaweed, and emended description of the genus *Gilvimarinus*

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A taxonomic study was carried out on strain YN3<sup>T</sup>, which was isolated from a seaweed sample taken from the coast of Weihai, China. The bacterium was Gram-stain-negative, rod-shaped, and could grow at pH 5.0–10.0 and 4–32 °C in the presence of 0–9.0% (w/v) NaCl. Strain YN3<sup>T</sup> was positive for the hydrolysis of polysaccharides, such as agar, starch and xylan. The predominant respiratory quinone was ubiquinone-8. The major fatty acids were C<sub>16:1</sub>ω7c and/or iso-C<sub>15:0</sub> 2-OH, C<sub>16:0</sub> and C<sub>18:1</sub>ω7c. The main polar lipids were diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine, and two unidentified glycolipids. The genomic DNA G + C content was 49.4 mol%. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain YN3<sup>T</sup> should be assigned to the genus *Gilvimarinus*. '*Gilvimarinus agarilyticus*' KCTC 23325 and *Gilvimarinus chinensis* QM42<sup>T</sup> had the closest phylogenetic relationship to strain YN3<sup>T</sup>, and showed 97.9% and 95.8% sequence similarities, respectively. On the basis of phenotypic, chemotaxonomic and genotypic data and DNA–DNA hybridization studies, we propose that strain YN3<sup>T</sup> represents a novel species of the genus *Gilvimarinus*, for which the name *Gilvimarinus polysaccharolyticus* sp. nov. is proposed. The type strain is YN3<sup>T</sup> (=KCTC 32438<sup>T</sup>=JCM 19198<sup>T</sup>). An emended description of the genus *Gilvimarinus* is also presented.

In 2009, the novel genus *Gilvimarinus* in the order *Alteromonadales* was proposed by Du *et al.* (2009) with *Gilvimarinus chinensis* as the type species, which was the only species with a validly published name in the genus *Gilvimarinus* until recently. *G. chinensis* QM42<sup>T</sup> could form hollow zones on an agar plate (Du *et al.*, 2009), and had agarase-encoding genes within its genome (NCBI accession nos WP\_02028688, WP\_020208740, WP\_020208752, WP\_020208794 and ARIX01000000 in BioProject PRJNA202777). Two years later, a novel member of the genus, '*Gilvimarinus agarilyticus*', was isolated from the seashore of Jeju Island. '*G.*

*agarilyticus*' could also degrade agar (Kim *et al.*, 2011). Many agarolytic bacteria have been isolated from marine environments, including seawater, marine sediments, seaweeds and some marine animal samples, such as sponges. These bacteria belong to many genera, including *Alterococcus* (Shieh & Jean, 1998), *Alteromonas* (Kirimura *et al.*, 1999; Leon *et al.*, 1992), *Agarivorans* (Du *et al.*, 2011; Long *et al.*, 2010), *Marinimicrobium* (Lim *et al.*, 2006), *Microbulbifer* (González *et al.*, 1997; Jeong *et al.*, 2013; Miyazaki *et al.*, 2008; Wang *et al.*, 2009; Zhang *et al.*, 2012), *Persicitalea* (Yoon *et al.*, 2007), *Pseudoalteromonas* (Schroeder *et al.*, 2003), *Rubritalea* (Scheuermayer *et al.*, 2006), *Simiduia* (Kim *et al.*, 2012a; Shieh *et al.*, 2008), *Saccharophagus* (Kim *et al.*, 2010), *Thalassomonas* (Jean *et al.*, 2006; Park *et al.*, 2011) and *Vibrio* (Aoki *et al.*, 1990; Macián *et al.*, 2001). Here, we describe a novel seaweed-associated agarolytic bacterium, strain YN3<sup>T</sup>,

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YN3<sup>T</sup> is HM437226.

Two supplementary figures are available with the online Supplementary Material.

isolated from the coast of Weihai in Shandong province, China.

Strain YN3<sup>T</sup> was originally recovered from a seaweed sample collected from the coast of Weihai, China. The samples were stored in darkness at 4 °C until micro-organisms were isolated. Dilutions of fragmented seaweed samples were spread on marine agar 2216 (MA; BD). After incubation at 25 °C for 7 days, one of the colonies was observed to form a clear hollow zone, which surrounded it on the plate. The colony was isolated and named strain YN3<sup>T</sup> as a pure culture after three successive transfers to fresh agar plates and was stored at -80 °C in 20% (v/v) glycerol.

Since strain YN3<sup>T</sup> propagated very well on MA but barely grew in marine broth 2216 (BD), tests of optimum growth conditions were taken on MA plates. The morphology of the colonies was observed on MA plates after 3–4 days of incubation at 28 °C. The morphology of the cells was examined by optical microscopy (BX40; Olympus) and transmission electron microscopy (JEM-1230; JEOL). The optimal NaCl concentration for growth was tested using NaCl-free MA plates with different NaCl concentrations (0, 1, 3, 5, 7, 9 and 10%, w/v). The temperature range for growth was determined by incubation at 4, 10, 15, 28, 32, 37, 42 and 50 °C on MA. The pH range for growth was determined on MA, which was adjusted to pH 5.0–10.0 (in 0.5 pH unit intervals) using appropriate biological buffers (MES for pH 5.0–6.5, PIPES for pH 6.5–7.0, Tricine for pH 7.0–9.0 and CAPSO for pH 9.0–10.0) at a concentration of 50 mM.

Cell motility of strain YN3<sup>T</sup> was assessed using a semi-solid stab-culture method according to MacFaddin (1976), with incubation at 28 °C for two weeks. The existence of the flagellum was confirmed by transmission electron microscopy. A few cells were washed and suspended in sterilized water, and the suspension was dropped onto a carbon-coated copper grid. The grid was stained with 2% (w/v) uranyl acetate and examined using a JEOL JEM-1230 transmission electron microscope at an accelerating voltage of 80 kV. Gram-stain reactions were tested according to *Bergey's Manual* (Holt *et al.*, 1994). Biomass for physiological and chemotaxonomic studies was obtained by cultivating the strains in modified marine broth with 0.1% (w/v) D-galactose and 0.5% (w/v) sucrose added for 3 days under optimum growth conditions. The catalase and oxidase activities and hydrolysis of DNA, gelatin, starch and Tweens 20, 40, 60 and 80 were determined according to Dong & Cai (2001). Hydrolysis of casein, chitosan, *p*-nitrophenyl butyrate (C4), L-tyrosine and xylan was determined on MA containing 2% (w/v) casein, 0.6% (w/v) chitosan, 0.3% (v/v) *p*-nitrophenyl butyrate, 0.5% (w/v) L-tyrosine and 0.6% (w/v) xylan. Additional enzyme activities and biochemical characteristics were tested using API ZYM, API 20 NE and API 50 CH test strips (bioMérieux) according to the manufacturer's instructions, except that cells were suspended in 2% (w/v) sea salts

(Sigma) for API 20 NE strips and in marine oxidation-fermentation medium (Leifson, 1963) for API 50 CH. Further tests of oxidation of various carbon sources were carried out using a Gram-negative GN2 MicroPlate (Biolog), according to the manufacturer's instructions, except that cells were suspended in 2% (w/v) sea salts (Sigma). Susceptibility to antibiotics was determined on MA 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), O/129 (10), penicillin G (10 IU), polymyxin B (300 U), streptomycin (10), sulfafurazole (300), sulfamethoxazole (1.25), tetracycline (30), trimethoprim (5) and vancomycin (30). The inhibition of cell growth by the antibiotics was observed after 7 days of incubation at 28 °C.

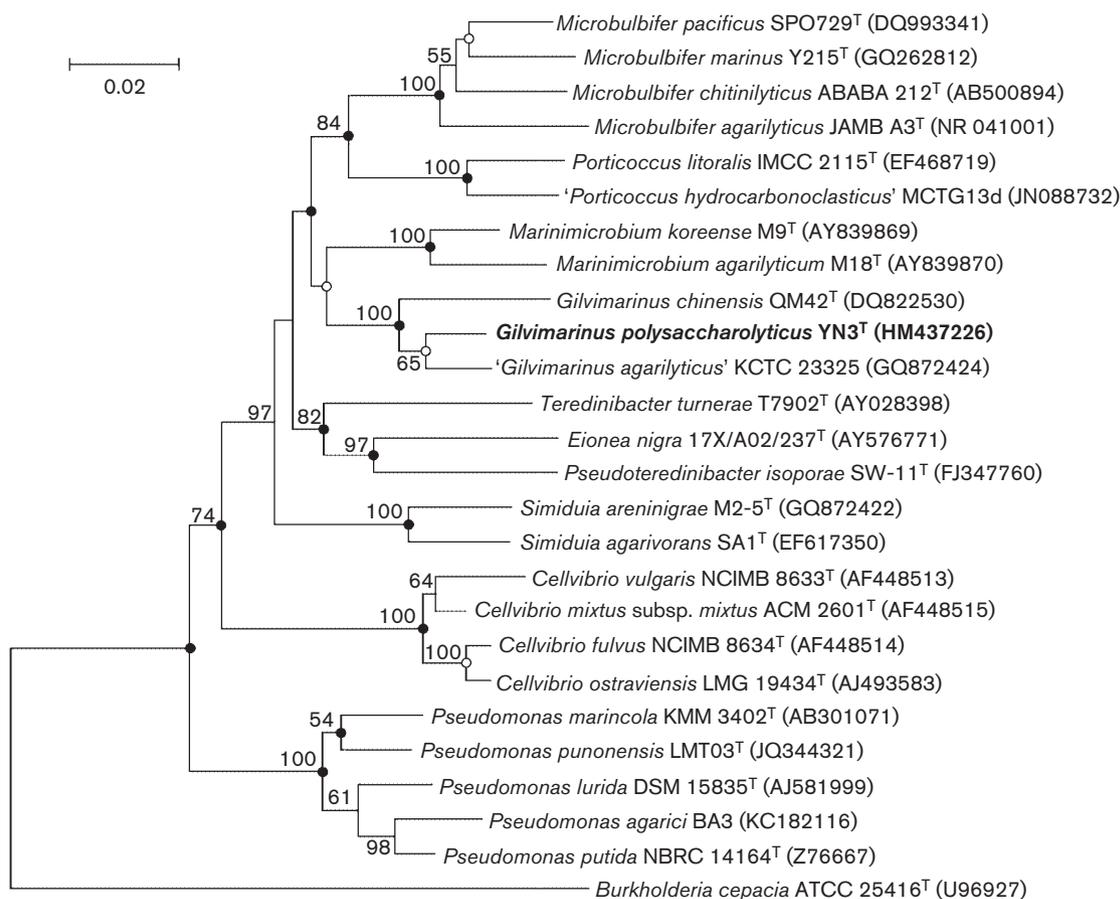
Genomic DNA was extracted according to the method described by Marmur & Doty (1961). The genomic DNA obtained was sequenced using next-generation sequencing technology (HiSeq2000; Illumina) with paired-end libraries of 500 bp and 6000 bp insert size. The DNA G + C content was calculated from the result of the whole genome sequence, and also determined by reversed-phase HPLC and calculated from the deoxyguanosine/thymidine ratio (Mesbah *et al.*, 1989). DNA-DNA hybridization experiments were performed using the thermal denaturation and renaturation method of De Ley *et al.* (1970) on a Beckman DU800 spectrophotometer. The hybridization temperature used was 71 °C and the experiments were carried out in triplicate. Total polar lipids were extracted and separated by two-dimensional TLC on silica gel plates (10 × 10 cm, no. 5554; Merck) according to the methods of Xu *et al.* (2007). Aminolipids were observed by spraying the plate with 0.5% ninhydrin in ethanol before heating it at 55 °C for 10 min. Phospholipids were observed after staining with molybdenum blue spray reagent (Sigma). Glycolipids were observed using the methods of Xin *et al.* (2000). The total lipids were detected by spraying 5% (w/v) phosphomolybdic acid in ethanol and heating at 140 °C for 10 min with ammonia. All the polar lipid images were further analysed as described by Minnikin *et al.* (1984). Cellular fatty acid methyl esters were obtained from freeze-dried cells, and analysed according to the instructions of the Sherlock Microbial Identification System (MIDI). Isoprenoid quinones were extracted from freeze-dried cells with chloroform/methanol (2 : 1, v/v) and identified by LC-MS (Tindall, 1990; Chung *et al.*, 1997).

Amplification of the almost-complete 16S rRNA gene was conducted via PCR using the universal primers 27F (5'-GAGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') (Winker & Woese, 1991). PCR products were cloned into vector pMD 19-T (TaKaRa) and sequenced by an automated DNA sequencer ABI 3730 (Applied Biosystems) with the ABI BigDye 3.1 sequencing kit (Applied Biosystems). The 16S rRNA

sequence was compared with recognized organisms using the EzTaxon-e service (Kim *et al.*, 2012b). The sequence dataset was aligned on the SINA Online service (Pruesse *et al.*, 2012), based on SILVA SSU/LSU databases. Phylogenetic trees were reconstructed using the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods with the MEGA 6 program (Tamura *et al.*, 2013). According to the best nucleotide substitution models found by the maximum-likelihood method in MEGA 6, the algorithm of the Jukes–Cantor model (Jukes & Cantor, 1969) was used to calculate the evolutionary distances, using the neighbour-joining and maximum-likelihood methods. The resultant tree topologies were evaluated by bootstrap analysis (Felsenstein, 1985) on the basis of 1000 replicates.

The 16S rRNA gene sequence (1503 nt) of strain YN3<sup>T</sup> was obtained. Strain YN3<sup>T</sup> showed the highest levels of 16S

rRNA gene sequence similarities with '*G. agarilyticus*' KCTC 23325 (97.9%) and *G. chinensis* QM42<sup>T</sup> (95.8%). The similarities shown by strain YN3<sup>T</sup> with the type strains of other species were all lower than 95%. Among those strains, the sequence similarities greater than 91% between strain YN3<sup>T</sup> and closely related genera were: *Marinimicrobium* (94.7–93.6%), *Cellvibrio* (93.8–92.0%), *Eionea* (93.7%), *Teredinibacter* (93.6%), *Saccharophagus* (93.4%), '*Candidatus Endobugula*' (93.1–91.7%), *Porticoccus* (92.9–92.8%), *Pseudoteredinibacter* (92.8%), *Simiduia* (92.8–92.4%), *Microbulbifer* (93.6–91.5%), *Dasania* (92.1%), *Umboniibacter* (92.25%) and *Pseudomonas* (91.3–91.0%). All the topological structures of neighbour-joining, maximum-likelihood and maximum-parsimony phylogenetic trees were identical in the coherent cluster of strain YN3<sup>T</sup>, '*G. agarilyticus*' KCTC 23325 and *G. chinensis* QM42<sup>T</sup>, indicating that strain YN3<sup>T</sup> belonged to the genus *Gilvimarinus* (Fig. 1).



**Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic relationships of the novel strain YN3<sup>T</sup> and related taxa. Open circles indicate that corresponding nodes were also identical in trees reconstructed using the maximum-likelihood method. Filled circles indicate that corresponding nodes were also recovered in the trees reconstructed using the maximum-likelihood and maximum-parsimony methods. Numbers at branch nodes refer to bootstrap values  $\geq 50\%$  (based on 1000 replicates). *Burkholderia cepacia* ATCC 25416<sup>T</sup> (GenBank accession no. U96927) was used as an out-group. Bar, 0.02 substitutions per nucleotide position.

**Table 1.** Differential characteristics of the novel strain YN3<sup>T</sup> with its closest phylogenetic relatives

Strains: 1, YN3<sup>T</sup>; 2, *G. chinensis* QM42<sup>T</sup>; 3, '*G. agarilyticus*' KCTC 23325. +, Positive; -, negative. All data were obtained from this study.

Characteristic	1	2	3
Growth at:			
4 °C	+	+	-
40 °C	-	+	-
0% NaCl (w/v)	+	-	-
Hydrolysis of:			
Tween 20	-	+	+
Tween 40	-	+	-
Tween 60	-	+	-
Tween 80	-	+	-
α-Galactosidase activity (API ZYM)	-	+	+
Utilization of (Biolog GN2):			
α-Cyclodextrin	+	-	+
N-Acetyl-D-glucosamine	-	+	+
L-Arabinose	-	-	+
D-Fructose	-	-	+
α-D-Glucose	-	+	+
α-Lactose	-	+	+
Lactulose	-	-	+
Melibiose	-	+	+
Trehalose	-	+	+
Turanose	-	+	-
Monomethyl succinate	+	-	-
D-Galacturonic acid	-	+	-
α-Ketovaleric acid	-	-	+
Antibiotic sensitivity			
Novobiocin (30 µg)	+	+	-
Penicillin G (10 IU)	-	+	-
Streptomycin (10 µg)	+	+	-
DNA G+C content (mol%) (by HPLC)	48.3	50.7	52.5

Cells of strain YN3<sup>T</sup> were Gram-stain-negative, aerobic and rod-shaped (0.4–1.0 µm in width and 1.0–3.0 µm in length). Cells showed motility with a single polar flagellum (Fig. S1, available in the online Supplementary Material). The other two strains, *G. chinensis* QM42<sup>T</sup> and '*G. agarilyticus*' KCTC 23325, both have single polar flagellum via transmission electron micrographs. All three strains can degrade agar on MA. The phenotypic characteristics of strain YN3<sup>T</sup> are given in detail in the species description. Strain YN3<sup>T</sup> showed significant differences from '*G. agarilyticus*' KCTC 23325 and *G. chinensis* QM42<sup>T</sup> in growth temperature. *G. chinensis* QM42<sup>T</sup> could grow at 40 °C while strain YN3<sup>T</sup> could not. Moreover, strain YN3<sup>T</sup> could grow at 4 °C but '*G. agarilyticus*' KCTC 23325 could not. Strain YN3<sup>T</sup> could survive without NaCl on MA, while '*G. agarilyticus*' KCTC 23325 and *G. chinensis* QM42<sup>T</sup> needed at least 1% (w/v) NaCl for growth. Moreover, strain YN3<sup>T</sup> could be distinguished from '*G. agarilyticus*' KCTC 23325 or *G.*

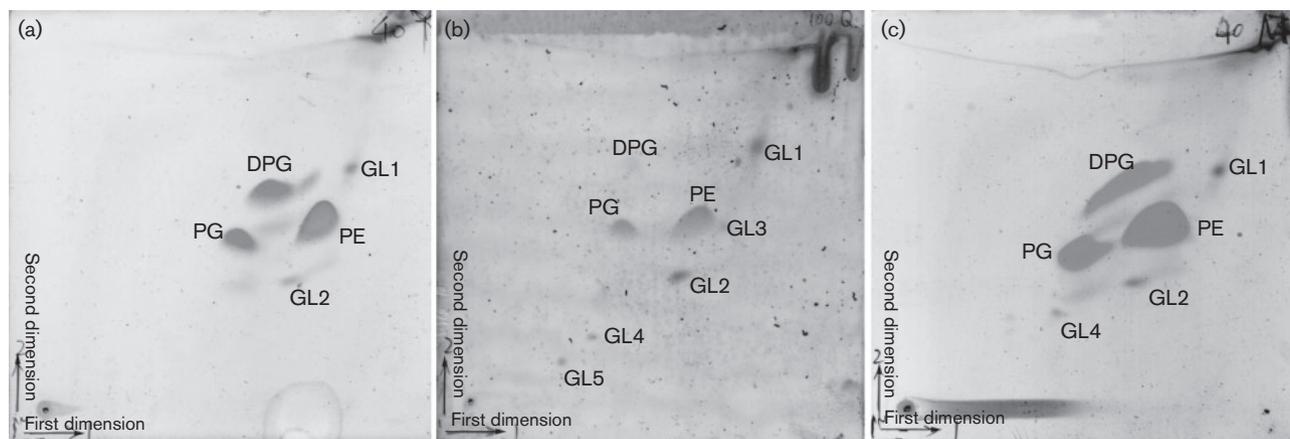
*chinensis* QM42<sup>T</sup> by some physiological characteristics, including hydrolysis of Tweens 20, 40, 60 and 80, activity of β-galactosidase and sensitivity to novobiocin, penicillin G and streptomycin. In addition, the utilization of succinic acid monomethyl ester was only observed in strain YN3<sup>T</sup>. The detailed differential properties of strains YN3<sup>T</sup>, '*G. agarilyticus*' KCTC 23325 and *G. chinensis* QM42<sup>T</sup> are listed in Table 1.

The DNA G + C content of strain YN3<sup>T</sup> was 48.3 mol% (by HPLC) and 49.4% (by whole genome sequencing). The DNA–DNA relatedness value of 38.1% between strain YN3<sup>T</sup> and reference strain '*G. agarilyticus*' KCTC 23325 was significantly below the threshold value of 70% (Wayne *et al.*, 1987). The main polar lipids of novel strain YN3<sup>T</sup> were diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine, and two unidentified glycolipids (GL1–2), among which the three kinds of phospholipids were identical to those of *G. chinensis* QM42<sup>T</sup> and '*G. agarilyticus*' KCTC 23325. However, there were only two unidentified glycolipids (GL1–2) found in strain YN3<sup>T</sup>, while there were five (GL1–5) in *G. chinensis* QM42<sup>T</sup> and three (GL1, 2 and 4) in '*G. agarilyticus*' KCTC 23325 (Fig. 2). This is the first report of polar lipid components of members of the genus *Gilvimirinus*. Further detailed polar lipid images with different specific stains are given in Fig. S2. The major cellular fatty acids (>10%) of strain YN3<sup>T</sup> were C<sub>16:1</sub>ω7c or iso-C<sub>15:0</sub> 2-OH (27.7%), C<sub>16:0</sub> (18.3%) and C<sub>18:1</sub>ω7c (12.3%), which are similar to those of '*G. agarilyticus*' KCTC 23325 and *G. chinensis* QM42<sup>T</sup>. Nevertheless, the contents of straight-chain fatty acids C<sub>15:0</sub>, C<sub>16:0</sub> and C<sub>17:0</sub> were greater in strain YN3<sup>T</sup> (7.8%, 18.3% and 6.3%) than in *G. chinensis* QM42<sup>T</sup> (2.6%, 14.7% and 3.8%) or '*G. agarilyticus*' KCTC 23325 (1.6%, 11.1% and 4.4%). However, the contents of hydroxy fatty acids C<sub>10:0</sub> 3-OH, C<sub>12:0</sub> 2-OH and C<sub>12:0</sub> 3-OH in strain YN3<sup>T</sup> (3.8%, 2.4% and 0.7%) were lower than those in '*G. agarilyticus*' KCTC 23325 (4.9%, 7.1% and 3.3%) and *G. chinensis* QM42<sup>T</sup> (6.1%, 4.5% and 4.7%). Summed feature 1 (iso-C<sub>15:1</sub> H and/or C<sub>13:0</sub> 3-OH) and summed feature 2 (containing one or more of unknown equivalent chain length 10.928, C<sub>12:0</sub> aldehyde, iso-C<sub>14:0</sub> 3-OH and/or iso-C<sub>16:1</sub> I) were only detected in strain YN3<sup>T</sup> (1.0% and 0.8%). Detailed fatty acid compositions of strain YN3<sup>T</sup>, '*G. agarilyticus*' KCTC 23325 and *G. chinensis* QM42<sup>T</sup> are listed in Table 2. The predominant respiratory quinone of strain YN3<sup>T</sup> was ubiquinone-8.

On the basis of physiological, chemotaxonomic and phylogenetic results from this study, strain YN3<sup>T</sup> is considered to represent a novel species within the genus *Gilvimirinus*, for which the name *Gilvimirinus polysaccharolyticus* sp. nov. is proposed.

**Emended description of the genus *Gilvimirinus* Du et al. 2009**

Cells are motile with a single polar flagellum. Cells can survive without NaCl and hydrolyse agar. The G + C



**Fig. 2.** Two-dimensional TLC of phospholipids and glycolipids of strain YN3<sup>T</sup> and reference strains. (a) Strain YN3<sup>T</sup>; (b) *G. chinensis* QM42<sup>T</sup>; (c) '*G. agarilyticus*' KCTC 23325. The plates were sprayed with 0.5% 1-naphthol in methanol/water (1:1, v/v) and then sulfuric acid/ethanol (1:1, v/v) (Xin *et al.*, 2000). DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; GL1–GL5, unknown glycolipid. All data were from this study.

content of the DNA varies between 48 and 53 mol%. The major quinone is ubiquinone-8. The major polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. The major cellular fatty acids are C<sub>16:1</sub>ω7c and/or iso-C<sub>15:0</sub> 2-OH, C<sub>16:0</sub> and C<sub>18:1</sub>ω7c. The rest of the description is identical to that mentioned by Du *et al.* (2009). The type species is *Gilvimirinus chinensis*.

### Description of *Gilvimirinus polysaccharolyticus* sp. nov.

*Gilvimirinus polysaccharolyticus* (po.ly.sac.cha.ro.ly'ti.cus. Gr. adj. *polus* many; Gr. n. *saccharon* sugar; N.L. masc. adj. *lyticus* from Gr. adj. *lutikos* -ē -on able to loosen, able to dissolve; N.L. masc. adj. *polysaccharolyticus* pertaining to the ability to hydrolyse polysaccharides).

Cells are rod-shaped (0.4–1.0 μm in width and 1.0–3.0 μm in length) under optimal growth conditions and Gram-stain-negative. Colonies are pale yellow, circular, slightly centrally sunken after 2 days of incubation at 28 °C on MA. Cells are motile with a single polar flagellum. Growth occurs at 4–32 °C with optimum temperature at 28–30 °C and pH 5.0–10.0 with the optimum at pH 7.0. Growth occurs in the presence of 0–9.0% (w/v) NaCl with optimum NaCl concentrations of 1.0–3.0%. Catalase- and oxidase-positive. Cells hydrolyse agar, DNA, starch and xylan, but not casein, chitosan, gelatin, L-tyrosine or Tweens 20, 40, 60 and 80. In API 20 NE tests, cells are positive for nitrate reduction and aesculin hydrolysis but negative for L-arginine, urea and 4-nitrophenyl β-D-galactopyranoside hydrolysis, indole production and D-glucose fermentation. In API ZYM tests, N-acetyl-β-glucosaminidase, acid phosphate, alkaline phosphatase, esterase (C4) and leucine arylamidase are positive; weakly positive activity is shown for α-glucosidase, β-glucosidase,

lipase (C14), naphthol-AS-BI-phosphohydrolase and valine arylamidase, while α-chymotrypsin, cystine arylamidase, esterase lipase (C8), β-fucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, α-mannosidase and trypsin are negative. In GN2 MicroPlates, cellobiose, maltose, monomethyl succinate, and Tween 80 are utilized, and the following substrates are weakly utilized: α-cyclodextrin, dextrin, glycogen, D-galactose, gentiobiose, D-mannose, methyl β-D-glucoside. Growth is not observed on N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, adonitol, L-arabinose, D-arabitol, i-erythritol, D-fructose, L-fucose, α-D-glucose, myo-inositol, α-lactose, lactulose, D-mannitol, melibiose, D-psicose, raffinose, L-rhamnose, D-sorbitol, sucrose, trehalose, turanose, xylitol, methyl pyruvate, acetic acid, cis-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, p-hydroxyphenylacetic acid, itaconic acid, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, DL-lactic acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebamic acid, succinic acid, bromosuccinic acid, succinamic acid, glucuronamide, L-alaninamide, D-alanine, L-alanine, L-alanyl-glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-pyrroglutamic acid, D-serine, L-serine, L-threonine, DL-carnitine, γ-aminobutyric acid, urocanic acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol, DL-α-glycerol phosphate, glucose 1-phosphate or glucose 6-phosphate. In API 50 CH assays, acid is produced from D-xylose, methyl β-D-xylopyranoside, D-galactose, D-glucose, D-fructose, D-mannose, methyl α-D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, aesculin (Fe<sup>3+</sup>), salicin, cellobiose, maltose, lactose, starch, glycogen,

**Table 2.** Fatty acid compositions of strain YN3<sup>T</sup> and its closest phylogenetic relatives

Strains: 1, YN3<sup>T</sup>; 2, *G. chinensis* QM42<sup>T</sup>; 3, '*G. agarilyticus*' KCTC 23325. —, Not detected. Data were obtained from this study under identical growth conditions. All three strains were grown for 3 days at 28 °C on modified marine broth. Values are percentages of total fatty acids.

Fatty acid	1	2	3
Straight-chain fatty acids			
C <sub>10:0</sub>	1.2	0.9	3.3
C <sub>11:0</sub>	1.1	0.8	1.1
C <sub>12:0</sub>	4.9	10.9	2.0
C <sub>13:0</sub>	1.0	0.5	0.1
C <sub>14:0</sub>	2.1	2.3	0.6
C <sub>15:0</sub>	7.8	2.6	1.6
C <sub>16:0</sub>	18.3	14.7	11.1
C <sub>17:0</sub>	6.3	3.8	4.4
C <sub>18:0</sub>	2.3	4.0	6.4
C <sub>19:0</sub>	0.1	—	0.1
Branched-chain fatty acids			
iso-C <sub>16:0</sub>	0.1	0.1	0.3
Unsaturated fatty acids			
C <sub>14:1</sub> ω5c	0.1	—	—
C <sub>15:1</sub> ω6c	0.2	0.1	0.1
C <sub>17:1</sub> ω8c	1.6	1.8	1.0
C <sub>17:1</sub> ω6c	0.3	—	0.3
C <sub>18:1</sub> ω9c	0.3	0.1	—
C <sub>18:1</sub> ω7c	12.3	11.0	15.4
C <sub>18:1</sub> ω6c	—	3.1	—
Hydroxy fatty acids			
C <sub>9:0</sub> 3-OH	0.2	0.1	0.1
C <sub>10:0</sub> 2-OH	0.3	0.6	—
C <sub>10:0</sub> 3-OH	3.8	6.1	4.9
C <sub>11:0</sub> 2-OH	0.8	0.7	0.2
C <sub>11:0</sub> 3-OH	0.6	0.8	1.4
C <sub>12:0</sub> 2-OH	2.4	4.5	7.1
C <sub>12:0</sub> 3-OH	0.7	4.7	3.3
C <sub>13:0</sub> 2-OH	0.2	—	0.5
C <sub>14:0</sub> 2-OH	—	—	0.1
Summed features*			
1	1.0	—	—
2	0.8	—	—
3	27.7	24.7	33.9
5	0.1	—	—
7	—	0.4	—

\*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 1 contains iso-C<sub>15:1</sub> H and/or C<sub>13:0</sub> 3-OH; summed feature 2 contains one or more of unknown equivalent chain length (ECL) 10.928, C<sub>12:0</sub> aldehyde, iso-C<sub>14:0</sub> 3-OH and/or iso-C<sub>16:1</sub> I; summed feature 3 contains C<sub>16:1</sub>ω7c and/or iso-C<sub>15:0</sub> 2-OH; summed feature 5 contains anteiso-C<sub>18:0</sub> and/or C<sub>18:2</sub>ω6,9c; summed feature 7 contains one or more of ECL 18.846, C<sub>19:0</sub> cyclo and/or C<sub>19:1</sub>ω6c.

gentiobiose, potassium 2-ketogluconate and potassium 5-ketogluconate. However, acid is weakly produced from glycerol, L-xylose, melibiose and trehalose, but not from erythritol, D-arabinose, L-arabinose, D-ribose, D-adonitol, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, sucrose, inulin, melezitose, raffinose, xylitol, turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol or potassium gluconate. Sensitive to amikacin, amoxicillin, ampicillin, carbenicillin, chloramphenicol, ciprofloxacin hydrochloride, erythromycin, gentamicin, kanamycin, nalidixic acid, neomycin, norfloxacin, novobiocin, polymyxin B, streptomycin, sulfafurazole, sulfamethoxazole, tetracycline, trimethoprim and vancomycin, but resistant to lincomycin, O/129 and penicillin G. The major cellular fatty acids (>10 %) include C<sub>16:1</sub>ω7c and/or iso-C<sub>15:0</sub> 2-OH, C<sub>16:0</sub> and C<sub>18:1</sub>ω7c. The predominant respiratory quinone is ubiquinone-8. The main polar lipids are diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine, and two unidentified glycolipids.

The type strain, YN3<sup>T</sup> (=KCTC 32438<sup>T</sup>=JCM 19198<sup>T</sup>), was isolated from a seaweed sample taken from the coast of Weihai, China. The genomic DNA G+C content is 49.4 mol% (determined by whole genome sequencing).

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