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Henriciella marina gen. nov., sp. nov., a Novel Member of the Family *Hyphomonadaceae* Isolated from the East Sea

Zhe-Xue Quan[1,2], Dan-Ning Zeng[1], Yi-Ping Xiao[1], Seong Woon Roh[3], Young-Do Nam[3], Ho-Won Chang[2], Jung-Hoon Yoon[2], Hee-Mock Oh[2], and Jin-Woo Bae[2,3]*

[1]*Department of Microbiology and Microbial Engineering, School of Life Sciences, Fudan University, Shanghai 200433, P. R. China*
[2]*Biological Resource Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon 305-333, Republic of Korea*
[3]*Department of Biology, Kyung Hee University, Seoul 130-701, Republic of Korea*

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A bacterial strain, designated Iso4^T, was isolated from the East Sea of Korea and was subjected to a polyphasic taxonomy study including phenotypic and chemotaxonomic characteristics as well as 16S rRNA gene sequence analysis. Cells of the strain were Gram-negative, motile, non-budding, non-stalked, and strictly aerobic. Strain Iso4^T grew optimally at 20°C in the presence of 1~2% (w/v) NaCl and at pH 6.9~7.6. The major respiratory quinone was Q-10 and the major cellular fatty acids were $C_{18:1}$ ω7c (53.5%), $C_{17:1}$ ω5c (11.7%), $C_{17:1}$ ω6c (8.1%), $C_{16:0}$ (7.8%), $C_{17:0}$ (4.8%), $C_{15:0}$ (2.9%), and $C_{16:1}$ ω5c (2.2%). The DNA G+C content of strain Iso4^T was 56.2 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain Iso4^T formed a monophyletic clade in the family *Hyphomonadaceae*, supported by high bootstrap value and was most closely related to the genus *Hyphomonas* (92~94%), a member of marine bacteria in the family. The phenotypic, genotypic, and chemotaxonomic evidences also suggest strain Iso4^T represents a novel genus and species in the family *Hyphomonadaceae*, for which the name *Henriciella* gen. nov., sp. nov. is proposed. The type strain is Iso4^T (=KCTC 12513^T =DSM 19595^T =JCM 15116^T).

***Keywords*:** *Henriciella marina* gen. nov., sp. nov., *Hyphomonadaceae*, taxonomy, East Sea

The family *Hyphomonadaceae*, which used to be a branch of *Rhodobacterales*, was suggested to be an extra group with *Caulobacterales* based on the 16S rRNA gene sequence analyses (Lee *et al.*, 2005) and the phylogenetic relationship of protein trees of the *Alphaproteobacteria* (Williams *et al.*, 2007). The family *Hyphomonadaceae* accomodates several genera: *Hyphomonas* (Moore *et al.*, 1984), *Hirschia* (Schlesner *et al.*, 1990), *Maricaulis* (Abraham *et al.*, 1999), *Oceanicaulis* (Strömpl *et al.*, 2003), and *Robiginitomaculum* (Lee *et al.*, 2007). Bacteria of this family, which were isolated mostly from marine environments, are generally Gram-negative, rod-shaped, chemo-organotrophic, and have dimorphic prosthecate cells during the incubation. This study aims to establish the taxonomic position of strain Iso4^T isolated from the East Sea based on a polyphasic taxonomy study. The isolate represents a distinct novel species of a novel genus, for which the name *Henriciella marina* is proposed.

Materials and Methods

Isolation and culture of bacterial strain

Strain Iso4^T was isolated from the coastal seawater in the East Sea of Korea at a depth of 100 m by a dilution-plating technique on marine agar 2216 (MA, Difco). The isolated colony was then incubated on MA at 30°C for further study. Bacterial cultures of the isolated strain were stored at -80°C in the presence of 20% (v/v) glycerol. Strain Iso4^T was submitted to the KCTC (Korean Collection for Type Cultures) as KCTC 12513^T, the DSMZ (German Collection of Microorganisms and Cell Cultures) as DSM 19595^T, and the JCM (Japan Collection of Microorganisms) as JCM 15116^T.

Phenotypic and biochemical characteristics

Cell morphology was examined after 5 days of incubation on MA at 30°C by light microscopy (Nikon) and transmission electron microscopy (EM912Ω, Leo Zeiss Inc.) after negative staining with 1% (w/v) phosphotungstic acid. Motility was determined by phase-contrast microscopy (Eclipse TS100, Nikon). The Gram reaction was determined using cells grown on MA at 30°C for 24 h according to the method described by Gerhardt *et al.* (1994). Anaerobic growth was determined on MA in an anaerobic test tube using the AnaeroGen kit (OXOID). Catalase activity was performed on bubble production in 3% (v/v) hydrogen peroxide solution. Oxidase activity was determined by oxidation of 1% (w/v) tetramethyl *p*-phenylenediamine (Merck). Growth at a variety of temperatures (4, 10, 15, 20, 25, 30, and 37°C) on MA and at different pH (5.3, 6.9, 7.6, 8.8, 9.3, 10.5, and 11.8) in Marine Broth (MB, Difco) was examined, respectively. Salt tolerance was tested in R2A broth in the presence of 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 15, 18, and 20% (w/v) NaCl (Reasoner and Geldreich, 1985). Enzyme activities and substrate utilization tests were conducted by using the API

* To whom correspondence should be addressed.
(Tel) 82-2-961-2312; (Fax) 82-2-961-0244
(E-mail) baejw@khu.ac.kr

ZYM and the API 20NE galleries (bioMérieux), respectively, according to the manufacturer's instructions.

Isoprenoid quinones and cellular fatty acids

Respiratory quinones were analyzed using reverse-phase HPLC as described by Komagata and Suzuki (1987). Cell biomass for fatty acid methyl ester (FAME) and quinone analysis was obtained from MA plates after 3 days of incubation at 30°C. For quantitative analysis of the cellular fatty acid composition, 40 mg wet cell materials were 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 analyzed by a gas chromatograph (Hewlett Packard 6890) and identified by the Microbial Identification software package (Sasser, 1990).

Determination of G+C content

Cell biomass for DNA extraction was obtained from MA plates at 30°C after 3 days of incubation. The isolation of chromosomal DNA was performed using a Cell Culture DNA Midi kit (QIAGEN, Canada) according to the manufacturer's instructions. The DNA G+C content was determined by using the fluorescence monitoring method (Xu *et al.*, 2000; Gonzalez and Saiz-Jimenez, 2002) with a LightCycler (Roche Diagnostics). The DNA of *Escherichia coli* B (Sigma-Aldrich, USA) was used as the calibration reference.

Determination of 16S rRNA gene sequencing and phylogenetic analysis

The 16S rRNA gene of strain Iso4^T was amplified by PCR using the universal primer pair 9F and 1512R as described previously (Quan *et al.*, 2005). The PCR product was purified with a QIAquick PCR Purification kit (QIAGEN) and sequenced using an Applied Biosystems 3730X1 DNA analyzer and the primers 519F, 536R, 907F, 1100R (Quan *et al.*, 2005). Full-length 16S rRNA gene sequences was compiled using SeqMan software (DNASTAR, USA). The identification of phylogenetic neighbors was preliminarily deposited by BLAST program. Other 16S rRNA gene sequences of these related taxa were obtained from GenBank (the accession numbers are given in Fig. 1). Multiple alignments were performed using the CLUSTAL X program (Thompson *et al.*, 1997). Gaps at the 5' and 3' ends of the alignment were omitted for further analysis. Phylogenic trees were constructed based on the neighbor-joining (Saitou and Nei, 1987), maximum-parsimony (Swofford, 1993), and minimum-evolution algorithms (Desper and Gascuel, 2002) using the MEGA 3 Program (Kumar *et al.*, 2004) with bootstrap values based on 1,000 replications (Felsenstein, 1985). The evolutionary distances were calculated using the method of Jukes and Cantor (1969).

Nucleotide sequence accession number

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Iso4^T is EF660760.

Results and Discussion

Phenotypic and biochemical characteristics

Cells of strain Iso4^T were Gram-negative, oxidase-positive, catalase-positive, motile, and rod-shaped. Compared with other bacteria in the family *Hyphomonadaceae* which can form prosthecae, stalked cells of strain Iso4^T were not observed by transmission electron microscopy during the culture. Colonies on MA were smooth, circular, translucent and shiny. Growth occurred at 10~37°C, pH 5.3~10.5 and in the presence of 1~15% NaCl. The enzyme activities were similar to the other members in the same family, i.e. positive for alkaline phosphatase, esterase lipase (C8) and

Table 1. Differential characteristics of strain Iso4^T and the type strains of related taxa in the family *Hyphomonadaceae*
Taxa: 1, Iso4^T (data from this study); 2, *Hyphomonas adhaerens* MHS-3^T (Weiner *et al.*, 2000); 3, *Hirschia balita* ATCC 49814^T (Schlesner *et al.*, 1990); 4, *Robiginitomaculum antarcticum* IMCC3195^T (Lee *et al.*, 2007); 5, *Oceanicaulis alexandrii* C116-18^T (Strömpl *et al.*, 2003); 6, *Maricaulis parjimensis* MCS 25^T (Abraham *et al.*, 2002); 7, *Woodsholea maritima* CM243^T (Abraham *et al.*, 2004). +, positive; -, negative; ND, not determined; v, various data

Characteristic	1	2	3	4	5	6	7
Cell morphology							
Shape	Ovoid or rod	Ovoid or Rod	Ovoid or rod	Vibrioid or rod	Vibrioid or rod	Vibrioid or fusiform	Vibrioid or rod
Prostheca	-	+	+	+	+	+	+
Hyphal	+	-	+	-	+	ND	-
Flagella	+	+	+	-	+	ND	+
Mode of division	Binary fission	Budding	Budding	Binary fission	Binary fission	Binary fission	Binary fission
Pigmentation	-	-	+	+	-	-	-
Optimum temperature (°C)	20	25~37	22~28	20	30	30~40	20~40
pH range for growth	5.3~10.5	5.7~8.7	ND	5.0~10.0	ND	ND	6.0~8.0
NaCl concentration (%) for growth	1.0~15.0	1.5~12.0	ND	0.5~5.0	2.0~10.0	0.5~10.0	0.5~10.0
Catalase	+	+	ND	+	+	ND	-
Oxidase	+	+	ND	-	+	ND	+
Nitrate reduction	-	+	-	+	+	v	-
DNA G+C content (mol%)	56.2	60.0	45.6	60.3	61.8	63.0	65.2

Table 2. Fatty acid contents (%) of strain Iso4^T and the type strains of related taxa in the family *Hyphomonadaceae*
Taxa: 1, Iso4^T (data from this study); 2, *Hyphomonas polymorpha* DSM 26653^T (Abraham *et al.*, 2004); 3, *Hirschia balita* ATCC 49814^T (Schlesner *et al.*, 1990); 4, *Robiginitomaculum antarcticum* IMCC 3195^T (Lee *et al.*, 2007); 5, *Oceanicaulis alexandrii* C116-18^T (Strömpl *et al.*, 2003); 6, *Maricaulis parjimensis* MCS 25^T (Abraham *et al.*, 2004); 7, *Woodsholea maritima* CM243^T (Abraham *et al.* 2004). -, not detected, trace amount (<1%)

Major fatty acid composition	1	2	3	4	5	6	7
$C_{12:0}$ 3-OH	1.1	tr	15.6	-	tr	-	3.5
$C_{15:0}$	2.9	1.9	2.2	tr	-	-	-
$C_{16:0}$	7.8	1.9	24.6	tr	1.8	3.6	1.4
$C_{16:1}$ ω5c	2.2	-	1.4	-	-	-	-
$C_{17:0}$	4.8	18.0	tr	14.3	9.9	7.0	2.2
$C_{17:1}$ ω5c	11.7	-	-	-	-	-	-
$C_{17:1}$ ω6c	8.1	15.0	-	7.7	1.1	1.8	tr
$C_{17:1}$ ω8c	-	11.0	-	21.4	-	4.7	-
$C_{17:0}$ iso	-	-	-	-	-	1.7	-
$C_{18:0}$	-	tr	2.1	3.5	22.1	7.9	16.9
$C_{18:1}$ ω7c	53.5	21.7	-	41.9	27.9	47.9	65.4
$C_{18:1}$ ω9c	-	-	-	2.8	-	6.0	-
$C_{18:1}$ ω11c	-	-	51.8	-	-	-	-
Summed feature 3[a]	1.7	-	-	-	-	-	2.7
ECL 18.797[b]	-	20.3	-	-	-	4.9	-

[a] Summed features consist of one or more fatty acids that could not be separated by the Microbial Identification System. Summed features 3: $C_{14:0}$ 3-OH, i$C_{16:1}$ I, ECL 10.968 and/or $C_{12:0}$ ALDE.
[b] Unidentified fatty acids with equivalent chain-length (ECL) given

leucine arylamidase activities; negative for α-galactosidase, β-galactosidase, α-glucuronidase, and α-mannosidase. However, strain Iso4^T showed distinct enzyme activities which were α-fucosidase positive but cystine arylamidase negative. Comparison of cultural, physiological and biochemical characteristics of strain Iso4^T with other members of the family *Hyphomonadaceae* are given in the species description and Table 1.

G+C content

The DNA G+C content of strain Iso4^T was 56.2 mol%, which falls in the range expected for members of the family *Hyphomonadaceae*. In addition, the G+C content of strain Iso4^T was lower than values of the most genera in the same family except *Hirschia* (45.6%).

Quinone composition and cellular fatty acids

Strain Iso4^T contained the characteristic ubiquinone of the family *Hyphomonadaceae*, Q-10. The fatty acids were $C_{18:1}$ ω7c (53.5%), $C_{17:1}$ ω5c (11.7%), $C_{17:1}$ ω6c (8.1%), $C_{16:0}$ (7.8%), $C_{17:0}$ (4.8%), $C_{15:0}$ (2.9%), $C_{16:1}$ ω5c (2.2%), summer feature 3 (1.7%), and $C_{12:0}$ 3-OH (1.1%). The high percent-

Table 3. API ZYM test of strain Iso4^T and the type strains of related taxa in the family *Hyphomonadaceae*
Taxa: 1, Iso4^T (data from this study); 2, *Hyphomonas polymorpha* DSM 26653^T (Abraham *et al.*, 2004); 3, *Robiginitomaculum antarcticum* IMCC 3195^T (Lee *et al.*, 2007); 4, *Oceanicaulis alexandrii* C116-18^T (Abraham *et al.*, 2004); 5, *Maricaulis parjimensis* MCS 25^T (Abraham *et al.*, 2004); 6, *Woodsholea maritima* CM243^T (Abraham *et al.*, 2004). All species are positive for alkaline phosphatase, esterase lipase (C8) and leucine arylamidase activities; all species are negative for α-galactosidase, β-galactosidase, α-glucuronidase, and α-mannosidase activities. +, positive; -, negative.

Biochemical activity	1	2	3	4	5	6
Enzyme essay						
Acid phosphatase	+	+	-	+	+	+
Esterase (C4)	+	+	-	+	+	+
Lipase (C14)	+	-	-	+	+	+
Valine arylamidase	+	-	+	+	+	+
Cystine arylamidase	-	+	+	+	+	+
Trypsin	+	-	-	+	+	+
α-Chymotrypsin	+	-	-	+	+	+
α-Glucosidase	+	+	-	-	+	-
β-Glucosidase	-	+	-	-	+	-
N-Acetyl-β-glucosaminidase	-	-	-	-	-	+
α-Fucosidase	+	-	-	-	-	-
Naphtol-AS-BI-phosphohydrolase	+	+	-	+	+	+

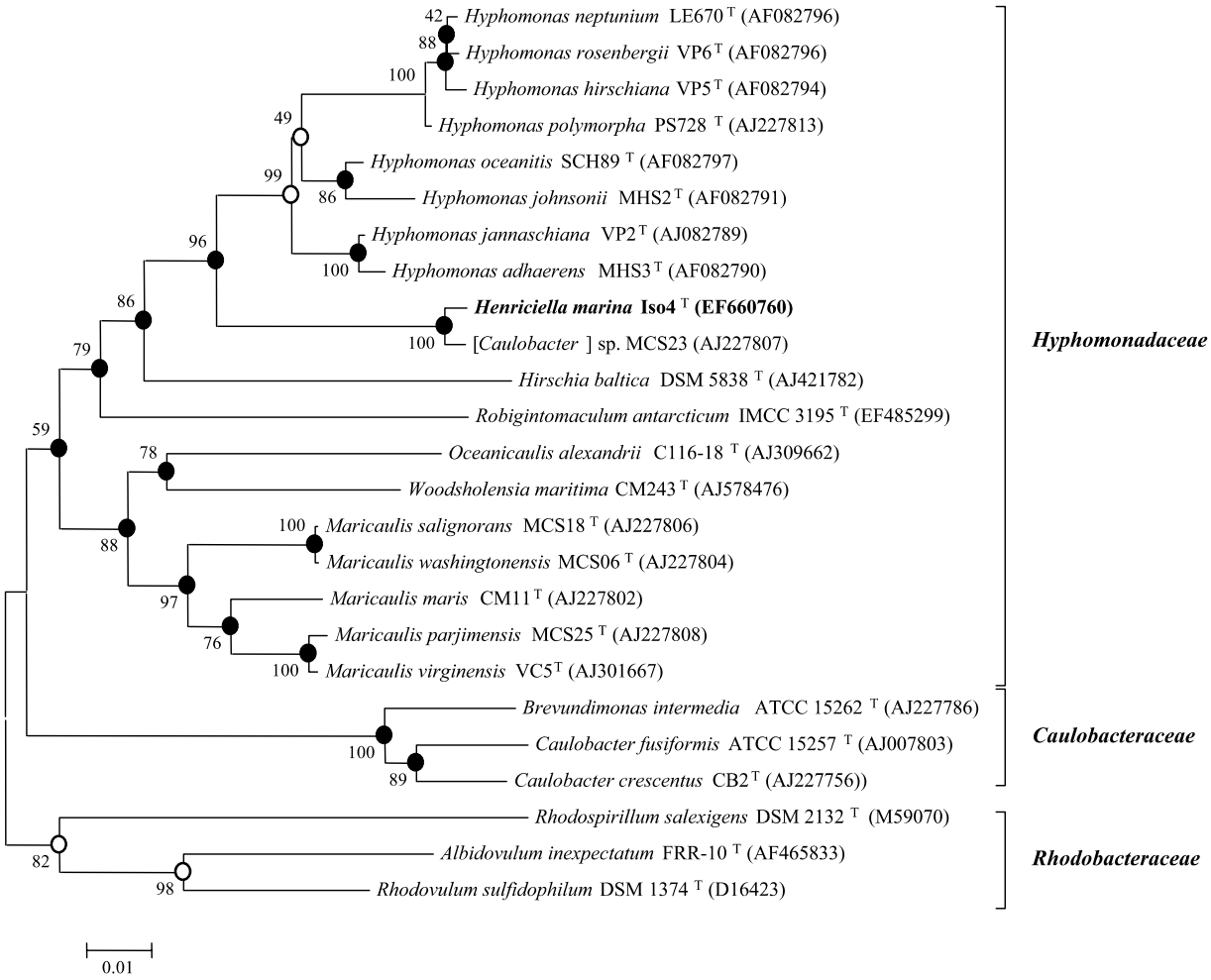

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationship between strain Iso4^T and related genera in the family *Hyphomonadaceae*. Closed dots represent the branches that were recovered by all three methods. Open circle indicate the branches that were covered by two method. Bootstrap values (expressed as percentages of 1,000 replications) of greater than 50% are shown at branch points. Bar, 1 substitution per 100 nucleotide positions.

age of fatty acid $C_{18:1}$ ω7c, shared by bacteria in the family *Hyphomonadaceae* except the genus *Hirschia*, suggested a close genetic relationship with other genera of the family *Hyphomonadaceae* (Table 2), whereas the relative predominant fatty acid $C_{17:1}$ ω5c showed moderate difference with other genera.

Phylogenetic analysis

The nearly complete 16S rRNA gene sequence of strain Iso4^T (1,391 bp) was obtained. Analysis of the 16S rRNA gene sequence similarities suggested that strain Iso4^T was closely related to the family *Hyphomonadaceae* in the class *Alphaproteobacteria*. [*Caulobacter*] sp. MCS23 (Abraham *et al.*, 1999) shared the highest similarity (99%) with strain Iso4^T. The novel strain was most closely related to the genera *Hyphomonas* (92%~94%), *Hirschia* (90%), *Maricaulis* (89%~90%), *Robiginitomaculum* (89%), *Oceanicaulis* (88%), and *Woodsholea* (88%). In the phylogenetic tree based on 16S rRNA gene sequences, strain Iso4^T formed a monophyletic clade in the family *Hyphomonadaceae* and was most related to the type strain of *Hyphomonas jannaschiana* (94%) (Fig. 1). The topologies of the maximum-parsimony and minimum-evolution trees were essentially the same, supported by high bootstrap value.

Taxonomic conclusions

On the basis of the relative low 16S rRNA gene sequence similarity and the large phylogenetic distance with the related type species in the family *Hyphomonadaceae*, combined with differential phenotypic and chemotaxonomic characteristics with its closest relatives, it was proposed that strain Iso4^T could be classified as a novel species of a new genus in the family *Hyphomonadaceae*, for which the name *Henriciella marina* gen. nov., sp. nov. is proposed.

We also found an extra genus *Woodsholea* (Abraham *et al.*, 2004), which was not included in the family *Hyphomonadaceae* by Lee *et al.* (2005), relatively shared a high similarity to *Oceanicaulis alexandrii* C116-18^T (93%). *Woodsholea maritima* CM243^T is a stalked marine bacterium, containing a relatively high percentage of a cellular fatty acid $C_{18:1}$ ω7c.

The major quinone of strain CM243^T is Q-10. The polyphasic data obtained from previous work (Abraham *et al.*, 2004) and phylogenetic analysis indicated that the genus *Woodsholea* formed an expanded clade of the family *Hyphomonadaceae*.

Description of *Henriciella* gen. nov.

Henriciella (Hen.ri.ci.el'la. N.L.fem. n. *Henriciella* named after Henrici, A.T., who first described stalked bacteria genus *Caulobacter*).

Cells are Gram-negative, aerobic, non-spore-forming, motile rods. Oxidase- and catalase-positive. Flagella are present. The major fatty acids are $C_{18:1}$ ω7c and C17:1 ω5c; the major respiratory quinone is Q-10. The DNA G+C content of type species is about 56 mol%. The genus is supposed to be a novel member of the family *Hyphomonadaceae*. The type species is *Henriciella marina*.

Description of *Henriciella marina* sp. nov.

Henriciella 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 0.4~0.7 μm wide and 0.7~2.3 μm long. Division mode is binary fission. Some cells form mycelium, ranging from 7.8 to 8.0 μm in length. Good growth occurs on R2A with 1% NaCl and MA. Colonies are translucent and shiny. Growth occurs at 10~37°C (optimum, 20°C), at pH 5.3~10.5 (optimum, pH 6.9~7.6), and in the presence of 1~15% NaCl (optimum, 1~2%). In the API ZYM system, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, trypsin, α-chymotrypsin, adn acid phosphatase, α-glucosidase, α-fucosidase, and naphthol-AS-BI-phosphohydrolase activities; negative for cystine arylamidase, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, and α-mannosidase *N*-acetyl-β-glucosaminidase activities. In the API 20NE system, negative for nitrate reduction, indole production, glucose acidification, β-galactosidase, and arginine dihydrolase activities; do not hydrolyze aesculin, urea, and gelatin; do not use glucose, arabinose, mannose, mannitol, *N*-acetyl-glucosamine, maltose, gluconate, caprate, adipate, malate, citrate, and phenyl-acetate as a sole carbon source. The major cellular fatty acids are $C_{18:1}$ ω7c (53.5%), $C_{17:1}$ ω5c (11.7%), $C_{17:1}$ ω6c (8.1%), $C_{16:0}$ (7.8%), $C_{17:0}$ (4.8%), $C_{15:0}$ (2.9%), $C_{16:1}$ ω5c (2.2%), summer feature 3 (1.7%), and $C_{12:0}$ 3-OH (1.1%).

The type strain Iso4^T (=KCTC 12513^T =DSM 19595^T =JCM 15116^T), was isolated from seawater in the East Sea of Korea, at a depth of 100 m.

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