# Jejuia spongiicola sp. nov., isolated from Callyspongia elongata<sup>§</sup>

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# Callyspongia elongata로부터 분리 된 신종 Jejuia spongiicola<sup>§</sup>

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A Gram-stain-negative, rod-shaped, non-flagella, non-gliding, obligate aerobic, and pale orange bacterium, strain 2012CJ34-3<sup>T</sup>, was isolated from a sponge sample of Callyspongia elongata from Chuja-myeon, Jeju-si, Jeju-do, Republic of Korea. On the basis of 16S rRNA gene sequencing, strain 2012CJ34-3<sup>T</sup> appeared closely related to Gaetbulibacter marinus KCTC 23081<sup>T</sup> (96.19%), *Gaetbulibacter lutimaris* KCTC 23716<sup>T</sup> (95.76%), Jejuia marina KCTC 42342<sup>T</sup> (95.70%), and Gaetbulibacter aquiaggeris KCTC 42198<sup>T</sup> (94.99%). Although the strain showed higher 16S rRNA gene similarity to Gaetbulibacter spp., phylogenetic analyses showed that it belongs to genus Jejuia. The average nucleotide identity and digital DNA-DNA hybridization between strain 2012CJ34-3<sup>T</sup> and Jejuia pallidilutea DSM 21165<sup>T</sup> were 75.0% and 19.20%, respectively. Growth occurs at 10-30°C on MA medium in the presence of 1-10% NaCl (w/v) and at pH 6.0-8.0. The DNA G + C content of the genomic DNA was 31.60 mol%, and menaquinone-6 (MK-6) was the major respiratory quinone. The major cellular fatty acids (> 5%) were  $C_{15:0}$  iso,  $C_{15:1}$  iso G,  $C_{15:0}$  iso 3OH and summed feature 3 ( $C_{16:1}$  $\omega 7c$  and/or C<sub>16:1</sub>  $\omega 6c$ ). The polar lipids consisted of phosphatidylethanolamine, unidentified aminophospholipid, unidentified aminolipid, unidentified glycolipid, and two unidentified lipids. Physiological and biochemical characteristics indicated that strain 2012CJ34-3<sup>T</sup> represents a novel species of the genus *Jejuia*, for which the name Jejuia spongiicola sp. nov. is proposed. The type strain is  $2012CJ34-3^{T}$  (= KACC  $22642^{T}$  = LMG  $32583^{T}$ ).

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<sup>§</sup>Supplemental material for this article may be found at http://www.kjom.org/main.html

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Porifera is the oldest evolutionary metazoan known to have originated from the Cambrian 600,000 years ago (White et al., 2012) and is a book called Sessile benthic invertebrate, which has evolved into a microorganism through filtration or microorganism. The sponges, called bacteriosponges or highmicrobial abundance (HMA) sponges, account for 40% of the living volume of symbiotic microorganisms, especially among various microbes that coexist in sponges, archaea, cyanobacteria, algae, red algae, and diatoms, and 26 species are known to exist (Cho and Park, 2009; Jackson et al., 2012). Symbiotic bacteria are reported to produce various industrially high-value natural products and physiological active substances, including immune mechanisms of sponges and formation of secondary metabolites while maintaining a permanent or temporary symbiotic relationship inside or outside the sponges (Selvin and Lipton, 2004; Kim and Dewapriya, 2012). The sponge symbiotic bacteria have been applied to natural product research and other applications. In addition, in the field of microbial ecology, attention has been focused on the symbiotic relationship between animals and microorganisms (Taylor et al., 2011).

The genus *Jejuia* resides with the family *Flavobacteriaceae*, which belongs to the class *Flavobacteriales* (Lee *et al.*, 2009). Members of the genus *Jejuia* commonly isolated from assorted

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marine environments, for example aquatic and seawater. All of them were generally Gram-negative, mesophilic, non-flagella, non-gliding and containing MK-6 as the major isoprenoid quinone (Lee *et al.*, 2009). Currently two *Jejuia* species have been described (http://www.bacterio.net/).

## Materials and Methods

#### Strain isolation

To screen for bacterial strains living in sponge from Callyspongia elongata in Chuja-myeon, Jeju-si, Jeju-do, Republic of Korea (33°59'25.0"N 126°14'58.6"E), sponge was collected from the sea at a depth of about 20-25 m using scuba diving and transferred to a laboratory for isolation. The environment in which Callyspongia elongata was collected general sea environment, and the water temperature at the time of collection was 16.3°C. The collected sponge was freeze-dried at -70°C for 24 h, and the freeze-dried sponge was ground in a sterilized mortar. The samples were carefully suspended in 0.85% (w/v) saline and carefully shaking saline containing powder sponge samples, the saline solution was diluted from  $10^{-1}$  to  $10^{-4}$ , spread on Marine agar medium (Difco) plates. Then, the plates were incubated at 25°C for 1 week. After 1 week, the strains were purified by subculturing on new MA plates. Five out of total 33 colonies were selected as novel species candidate species, and Ruegeria, Jejuia, Flagellimonas, Muricauda, and Sansalvadorimonas genera were identified. Among them Jejuia sp. 2012CJ34-3<sup>T</sup> was routinely cultured on MA agar and maintained in a glycerol suspension (Marine broth with 20%, v/v), at -80°C.

#### Information on reference strains

In this current report, we describe a novel bacterial strain, designated 2012CJ34-3<sup>T</sup>, which appears to be a member of the genus *Jejuia*. Reference strains (*Jejuia marina* KCTC 42342<sup>T</sup>, *Gaetbulibacter lutimaris* KCTC 23716<sup>T</sup>, *Gaetbulibacter aquiaggeris* KCTC 42198<sup>T</sup>, and *Gaetbulibacter marinus* KCTC 23081<sup>T</sup>) were obtained respectively from Korean Collection for the Type Cultures (KCTC) for use in a comparative analysis.

# Morphological, physiological and biochemical characterization

The Gram staining was determined using the described method of Buck (1982). Cell shape, size, and the presence of flagella were determined under a LIBRA 120 (120 kV) transmission electron microscope (Carl Zeiss) and Nikon light microscopy (×1000 magnification), after cells grown for 3 days at 25°C on MA medium. Motility was checked on Marine broth supplemented with 0.2% agar (Weon et al., 2008). Cell growth of strain 2012CJ34-3<sup>T</sup> was monitored at various temperatures 4, 10, 18, 25, 30, 37, 42, 45, 50°C, respectively. Various initial pH values (4-10 at intervals of 0.5 pH units) evaluated after 7 days of incubation at 25°C using Marine broth. The following buffers (each 20 mM final concentration) were used to adjust the pH of marine broth: acetate buffer for pH 4.0-5.5, phosphate buffer for pH 6.0-8.0 and Tris buffer for pH 8.5-10.0. Salt tolerance was tested in marine broth that controlled only the concentration of sodium chloride in the composition of the marine medium supplemented with 0.5% to 10% (w/v at intervals of 1% unit) NaCl and growth assessed after 7 days of incubation at 25°C. An anaerobic growth test was conducted with the GasPak<sup>TM</sup> EZ anaerobe pouch system (BD) over two weeks. Tests for the hydrolysis of Tween-60, casein, starch, carboxyl methyl cellulose (Barrow and Feltham, 1974; Atlas, 1993), DNA (using DNase agar from Scharlau, with DNase activity detected by flooding plates with 1 M HCl) were carried out after 5 days of incubation at 25°C. Biochemical tests were carried out using commercial API (API 20NE, API ID 32GN, and API ZYM) kits according to the manufacturer (bioMérieux) instructions. The API ZYM test strip was read after 4 h of incubation at 37°C, and the other API strips were examined after 2 days at 25°C. Catalase and oxidase activities were determined as previously described (Cappuccino and Sherman, 2002).

### Phylogenetic analysis

Genomic DNA of strain 2012CJ34-3<sup>T</sup> was isolated using a genomic DNA extraction kit (Macrogen Co. Ltd.) for 16S rRNA sequence and genome sequence, and the 16S rRNA gene was amplified using the universal bacterial primer set (800R, 1492R, 27F, and 518F) (Lane, 1991). Then, the purified PCR products were sequenced by Macrogen Co. Ltd. The sequence of the 16S rRNA gene was compiled using SeqMan software

(DNASTAR) and the 16S rRNA gene sequences of related taxa, which were obtained from the GenBank database and EzTaxon-e server (http://www.ezbiocloud.net) (Yoon *et al.*, 2017a). Multiple alignments were performed by Clustal\_X program with gaps edited in BioEdit program (Thompson *et al.*, 1997; Hall, 1999). Maximum-likelihood (ML), maximum-parsimony (MP), and neighbor-joining (NJ), trees were constructed using the Molecular Evolutionary Genetics Analysis 11 (MEGA 11.0) software with bootstrap analysis based on 1,000 replications. Kimura two parameter model was used for the ML and NJ tree construction with pairwise deletion of gaps, while, MP tree was made with Subtree-Pruning-Regrafting heuristic method with gaps of pairwise deletion (Fitch, 1971; Kimura, 1980; Felsenstein, 1985; Saitou and Nei, 1987; Kumar *et al.*, 2016).

#### Draft genome sequencing and G + C content analysis

The minimal standards for use of genome data in taxonomy of prokaryotes leaded these analyses (Chun et al., 2018). The draft genomic sequencing of strain 2012CJ34-3<sup>T</sup> was performed by Illumina HiSeq X Ten analysis and assembled using the SOAPdenovo v. 3.10.1 de novo assembler. The draft genome sequence was submitted to the GenBank database (www.ncbi. nlm.nih.gov) and annotated using the NCBI Prokaryotic Genomes Annotation Pipeline (PGAP) (Tatusova et al., 2016), from which the genomic DNA G+C content was determined directly. Pairwise genome-based relatedness between strain 2012CJ34-3<sup>T</sup> and closely related strain, Jejuia pallidilutea DSM 21165<sup>T</sup> (GCA 002973595) were estimated based on the average nucleotide identity (ANI) using the ANI calculator employing the OrthoANIu algorithm (Yoon et al., 2017b) available from the EzBioCloud service. The digital DNA-DNA hybridization (dDDH) value was calculated using the online Genome to Genome Distance Calculator (http://ggdc.dsmz.de/ggdc.php) (Li et al., 2019).

#### Chemotaxonomic analysis

Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), evaporated under vacuum conditions, and reextracted in n-hexane/water (1:1, v/v). The crude n-hexane-quinone solution was purified using Sep-Pak Vac cartridges silica (Waters) and subsequently analyzed by HPLC as previously described (Hiraishi *et al.*, 1996). Cellular fatty acids profiles

were determined for strains grown on MA medium for 2 days at  $30^{\circ}$ C. The cellular fatty acids were saponified, methylated, and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). The fatty acid methyl esters were then analysed by gas chromatography (model 6890; Hewlett Packard) using the Microbial Identification software package (Sasser, 1990). Strain 2012CJ34-3<sup>T</sup> was examined for their polar lipid contents as described previously (Minnikin *et al.*, 1984).

## Results

#### Physiological characteristics

Colonies of strain 2012CJ34-3<sup>T</sup> grown on MA agar plates for 2 days at 25°C were smooth, convex, circular with entire margins, pale orange colored. Cells were Gram-staining-negative, obligate aerobic, non-spore-forming, devoid of flagellar and gliding motility, and rod-shaped (0.3–0.7  $\mu$ m in diameter and 1.2–2.7  $\mu$ m in length) (Fig. 2). It was positive for cellulose but not casein, starch, Tween 80, and DNA. It grew at occurs at 10– 30°C (optimum, 25°C), at pH 6.0–8.0 (optimum, pH 7.0) and in the presence of 0.0–10.0% (w/v) NaCl (optimum, 2.0%). Furthermore, the physiological and biochemical characteristics of strain 2012CJ34-3<sup>T</sup> are summarized in the description and Table 1.

#### Phylogenetic tree analysis

The complete 16S rRNA gene sequence (1,453 bp) of strain 2012CJ34-3<sup>T</sup> was determined and subjected to a comparative analysis. The comparison indicated highest sequence similarity to *Gaetbulibacter marinus* KCTC 23081<sup>T</sup> (96.19%), *Gaetbulibacter lutimaris* KCTC 23716<sup>T</sup> (95.76%), *Jejuia marina* KCTC 42342<sup>T</sup> (95.70%), and *Gaetbulibacter aquiaggeris* KCTC 42198<sup>T</sup> (94.99%). Although the strain showed higher similarities to *Gaetbulibacter* spp., maximum likelihood tree (Fig. 1) and neighbor joining tree (Supplementary data Fig. S1) showed that the strain forms a monophyletic clade with all two known species of genus *Jejuia* supported by moderate bootstrap values (58 and 56, respectively). Based on 16S rRNA gene sequence and phylogenetic tree analyses, these strains were used as reference strains in most of the phenotypic analyses.

**Table 1.** Physiological and biochemical characteristics between strain 2012CJ34-3<sup>T</sup> and closely related species of the genus *Jejuia* and *Gaetbulibacter* Strains: 1, *Jejuia spongiicola* 2012CJ34-3<sup>T</sup>; 2, *Jejuia marina* KCTC 42342<sup>T</sup>; 3, *Gaetbulibacter lutimaris* KCTC 23716<sup>T</sup>; 4, *Gaetbulibacter aquiaggeris* KCTC 42198<sup>T</sup>; 5, *Gaetbulibacter marinus* KCTC 23081<sup>T</sup>.

All tests were obtained in this study. All strains are positive for alkaline phosphatase, esterase (C4), leucine arylamidase, valine arylamidase, acid phosphatase and naphtol-AS-BI-phosphohydrolase. Negative for nitrate reduction, indole production, glucose acidification, arginine dihydrolase, urease, L-arabinose, N-acetyl-D-glucosamine, caprate, adipate, malate, citrate, phenyl-acetate, salicin, D-melibiose, L-fucose, D-sorbitol, valerate, L-histidine, 2-ketogluconate, 3-hydroxy-butyrate, 4-hydroxy-benzoate, L-rhamnose, D-ribose, inositol, itaconate, suberate, malonate, lactate, L-alanine, 5-ketogluconate, glycogen, 3-hydroxy-benzoate,  $\alpha$ -galactosidase,  $\beta$ -glucuronidase and  $\alpha$ -mannosidase. +, positive; w, weakly positive; -, negative.

Characteristics	1	2	3	4	5
Isolation source	Sponge	Beach <sup>a</sup>	Sediment <sup>b</sup>	Sea water <sup>c</sup>	Sea water <sup>d</sup>
Pigmentation	Pale orange	Pale yellow <sup>a</sup>	Strong orange yellow <sup>b</sup>	Yellow <sup>c</sup>	Yellow <sup>d</sup>
API 20 NE & ID 32 GN tests					
β-Glucosidase (esculin hydrolysis)	-	-	+	+	+
Protease (gelatin hydrolysis)	+	-	-	-	-
β-Galactosidase (PNPG)	-	-	-	+	-
D-Glucose	+	+	W	-	+
D-Mannose	-	+	W	-	+
D-Mannitol	-	W	-	-	-
D-Maltose	-	W	-	-	+
Gluconate	-	W	-	-	-
Propionate	-	-	-	-	+
L-Proline	-	-	-	-	+
D-Sucrose	-	-	-	-	+
Acetate	+	-	-	-	+
L-Serine	-	-	-	-	+
API ZYM results					
Esterase lipase (C8)	W	+	+	+	+
Lipase (C14)	-	+	-	-	-
Cystine arylamidase	-	+	+	W	+
Trypsin	+	-	-	-	-
α-Chymotrypsin	-	+	-	+	+
α-Glucosidase	-	+	W	+	+
N-Acetyl-β-glucosaminidase	+	-	-	+	-
x-Fucosidase	+	-	W	-	-
G + C content (mol%)	31.6	34.2ª	34.6 <sup>b</sup>	36°	38.1 <sup>d</sup>

\*Data from <sup>a</sup> Kim et al. (2015); <sup>b</sup> Yoon et al. (2013); <sup>c</sup> Jung et al. (2016); <sup>d</sup> Yang and Cho (2008).

<sup>†</sup>The DNA G + C content of strain 2012CJ34-3<sup>T</sup> was calculated from its genome.

### Draft genome sequencing and G + C content analysis

The genome of strain 2012CJ34-3<sup>T</sup> consists of a circular chromosome with 3,949,721 bp and a G + C content of 31.60 mol%, consisting of 45 contigs with an N50 value of 598,647 bp. The average sequencing depth of coverage was 1254.56X determined. The 16S rRNA gene sequence made using Sanger sequencing methods was 100% identical to those gene extracted from the 2012CJ34-3<sup>T</sup> annotated genome. The genome includes 3,406 coding genes (CDSs), 6 rRNAs, 45 tRNAs and 4 ncRNAs

genes. According to the genome annotation based on RAST (Aziz *et al.*, 2008), nitrogen metabolism genes were encoded in the genome of the strain 2012CJ34-3<sup>T</sup>, but the motility-related genes were not encode (Supplementary data Fig. S2). The average nucleotide identity and digital DNA-DNA hybridization between strain 2012CJ34-3<sup>T</sup> and *Jejuia pallidilutea* DSM 21165<sup>T</sup> were 75.0% and 19.20%, respectively, which were below the proposed ANI cut-off values of 95–96% and dDDH cut-off values of 70% for interspecies identity (Goris *et al.*, 2007).

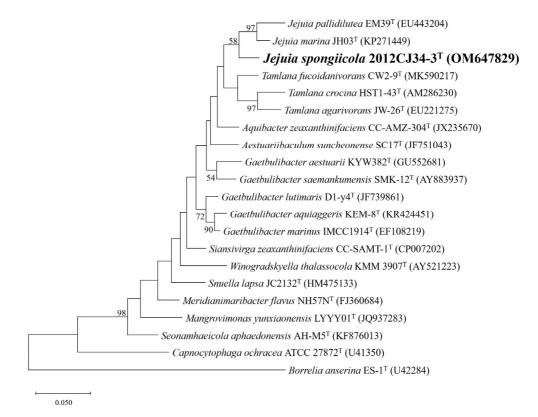


Fig. 1. Maximum-likelihood phylogenetic tree constructed from a comparative analysis of 16S rRNA gene sequences showing the relationships of 2012CJ34-3<sup>T</sup> with other related species of the genus *Jejuia*. Bootstrap values (expressed as percentages of 1,000 replications) greater than 50% are shown at the branch points. Bar, 0.02 substitutions per nucleotide position.

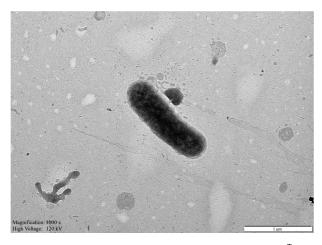


Fig. 2. Transmission electron micrograph of strain 2012CJ34-3<sup>T</sup>. Bar represents 1  $\mu$ m.

#### Chemotaxonomic analysis

The major quinone detected in strain  $2012CJ34-3^{T}$  was menaquinone-6 (MK-6), which is same to other species in genus *Jejuia*. The major cellular fatty acids profiles of strain

2012CJ34-3<sup>T</sup> were mainly composed of  $C_{15:0}$  iso (21.79%),  $C_{15:1}$  iso G (18.77%),  $C_{15:0}$  iso 3OH (12.9%) and  $C_{16:1} \omega 7c$  and/or  $C_{16:1} \omega 6c$  (summed feature 3) (12.9%), which is similar to those of described species in the genus *Jejuia* (Table 2). The major polar lipid was phosphatidylethanolamine (PE), unidentified aminophospholipid (APL), unidentified aminolipid (AL), unidentified glycolipid (GL) and two unidentified lipids (Ls) (Fig. 3). From the polar lipid analysis, the novel isolate was found to share major polar lipid PE with described species in the genus *Jejuia*.

## Discussion

Based on our taxonomic and morphological analyses, strain 2012CJ34-3<sup>T</sup> shares major MK-6 as menaquinone, and  $C_{15:0}$  is,  $C_{15:1}$  iso G,  $C_{15:0}$  iso 3OH and  $C_{16:1} \omega 7c$  and/or  $C_{16:1} \omega 6c$  (summed feature 3) as major fatty acids (CFAs) and phosphatidyle-thanolamine (PE), as major polar lipids with described species

#### Table 2. Fatty acid profiles of strain 2012CJ34-3<sup>T</sup> and related species of the genus *Jejuia* and *Gaetbulibacter*

Strains: 1, *Jejuia spongiicola* 2012CJ34-3<sup>T</sup>; 2, *Jejuia marina* KCTC 42342<sup>T</sup>; 3, *Gaetbulibacter lutimaris* KCTC 23716<sup>T</sup>; 4, *Gaetbulibacter aquiaggeris* KCTC 42198<sup>T</sup>; 5, *Gaetbulibacter marinus* KCTC 23081<sup>T</sup>.

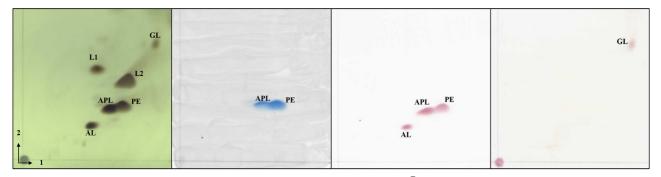
All strains were cultured on MA medium for 48 h at 25°C. Some fatty acids amounting to < 0.5% of the total fatty acids in all strains are not listed. tr, trace amounting (< 0.5%); –, not detected.

Fatty acid	1	2	3	4	5
Saturated					
C <sub>12:0</sub>	0.9	TR	TR	TR	TR
C <sub>14:0</sub>	0.9	TR	TR	0.6	0.6
C <sub>16:0</sub>	4.3	2.3	1.7	1.3	1.7
Unsaturated					
$C_{15:1} \omega 6c$	TR	TR	0.63	TR	TR
C <sub>17:1</sub> <i>w</i> 6 <i>c</i>	TR	0.7	1.3	0.9	0.6
$C_{17:1} \omega 8c$	TR	TR	0.6	0.5	TR
$C_{18:1} \omega 9c$	TR	0.6	TR	TR	TR
Branched-chain fatty acid					
C <sub>13:0</sub> iso	0.6	TR	0.8	1.1	0.5
C <sub>14:0</sub> iso	1.0	1.6	1.6	4.0	2.2
C <sub>14:0</sub> iso 3OH	TR	0.7	TR	TR	0.6
C <sub>14:1</sub> iso E	TR	TR	TR	1.1	0.5
C <sub>15:0</sub> iso	21.8	11.7	12.6	15.8	14.6
C <sub>15:0</sub> iso 3OH	12.9	5.2	8.7	5.5	4.2
C <sub>15:1</sub> iso G	18.8	13.6	10.9	14.2	21.8
C <sub>16:0</sub> iso	1.6	2.0	1.2	3.0	3.9
C <sub>16:0</sub> iso 3OH	2.3	13.9	5.6	12.9	13.2
C <sub>16:1</sub> iso G	1.0	1.7	TR	TR	2.4
C <sub>16:1</sub> iso H	TR	TR	1.0	1.7	TR
C <sub>17:0</sub> iso 3OH	12.9	9.6	14.2	9.4	8.4
C <sub>15:0</sub> anteiso	2.9	13.2	15.4	7.8	6.2
C <sub>15:1</sub> anteiso A	2.2	5.7	4.7	1.3	5.2
C <sub>17:1</sub> anteiso A	TR	TR	1.78	TR	TR
$C_{17:1}$ anteiso $\omega 9c$	TR	TR	TR	TR	0.8
Hydroxy fatty acids					
C <sub>15:0</sub> 2OH	1.3	2.3	2.8	1.7	1.1
C <sub>15:0</sub> 3OH	0.6	0.9	TR	1.4	TR
C <sub>16:0</sub> 3OH	1.1	1.3	0.8	1.4	0.9
C <sub>17:0</sub> 2OH	0.9	5.2	4.7	1.0	1.9
Other					
C <sub>13:1</sub> at 12-13	TR	TR	TR	0.74	TR
Summed feature					
<b>3;</b> C <sub>16:1</sub> ω7 <i>c</i> /C <sub>16:1</sub> ω6 <i>c</i>	8.4	4.2	4.2	9.9	4.8
<b>9</b> ; C <sub>17:1</sub> iso ω7 <i>c</i> /C <sub>16:0</sub> 10-methyl	1.3	TR	1.8	0.8	0.8

\*Summed features are fatty acids that cannot be resolved reliably from another fatty acid using the chromatographic conditions chosen. The MIDI system groups these fatty acids together as one feature with a single percentage of the total.

in the genus *Jejuia*. The phylogenetic trees based on 16S rRNA gene sequences places 2012CJ34-3<sup>T</sup> in genus *Jejuia* and the low similarity ( $\leq$ 95.70) the novel strain represent novel species in the genus. Chemotaxonomic and phenotypic characteristics

supports that it belongs to *Jejuia* and differentiate the novel isolate from *Jejuia* species (Tables 1 and 2). Hence, strain 2012CJ34-3<sup>T</sup> represents a novel species in the genus *Jejuia* for which the name *Jejuia spongiicola* sp. nov. is proposed.



**Fig. 3. Two-dimensional thin-layer chromatography of the total polar lipids of strain 2012CJ34-3<sup>T</sup>.** Chloroform-methanol-water (65:25:4, by vol.) was used in the first direction, followed by chloroform-acetic acid-methanol-water (40:7.5:6:2, by vol.) in the second direction. The following spray reagents were used for detection: (a) 5% ethanoic molybdophosphoric acid (for total lipids); (b) molybdenum blue (Sigma) (for phosphorus-containing polar lipids); (c) 2% ninhydrin (for amino lipids); (d) *a*-naphthol (Sigma) (for glycolipis). PE, phosphatidylethanolamine; APL, unidentified aminophospholipid; AL, unidentified aminolipid; GL, unidentified glycolipid; L, unidentified lipids.

#### Description of Jejuia spongiicola sp. nov.

*Jejuia spongiicola* (spon.gi.i'co.la. L. fem. n. *spongia*, sponge; L. masc./fem. n. suff. *-cola*, inhabitant, dweller; from L. masc./fem. n. *incola*, dweller; N.L. masc./fem. n. *spongiicola*, sponge inhabitant).

Cells are Gram-stain-negative, obligate aerobic, catalase positive but not oxidase. Colonies grown on MA are smooth, convex, circular with entire margins, 0.4-1.1 mm in diameter and pale orange colored. Growth occurs at 10-30°C (optimum, 25°C) in the presence of 0.0-10.0% NaCl (w/v, optimum 2.0%) and at pH 6.0-8.0 (optimum, pH 7.0), although not on R2A agar, TSA, LB agar, nutrient agar. Positive for the hydrolysis of cellulose but not casein, DNase, starch, and tween-80. Details of carbon assimilation (API ID 32GN and API 20NE) and enzyme activities (API ZYM) are listed in Table 1. The predominant quinone is MK-6. The major cellular fatty acids are C<sub>15:0</sub> is, C<sub>15:1</sub> iso G, C<sub>15:0</sub> iso 3OH and C<sub>16:1</sub> ω7c and/or  $C_{16:1} \omega 6c$  (summed feature 3). The polar lipids are phosphatidylethanolamine (PE), unidentified aminophospholipid (APL), unidentified aminolipid (AL), unidentified glycolipid (GL) and two unidentified lipids (Ls). The DNA G+C content of genomic DNA is 31.60 mol%.

The type strain,  $2012CJ34-3^{T}$  (= KACC  $22642^{T}$  = LMG  $32583^{T}$ ) was isolated from *Callyspongia elongata* in Chujamyeon, Jeju-si, Jeju-do, Republic of Korea, and has been deposited in the MABIK (National Marine Biodiversity Institute of Korea) with the number MPRBM-20210617003.

The draft genome and 16S rRNA gene sequence of strain 2012CJ34-3<sup>T</sup> has been deposited at GenBank/EMBL/DDBJ

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under accession numbers JAMFLZ00000000 and OM647829, respectively.

# 적 요

대한민국, 제주시, 추자면에서 얻어진 해면 Callyspongia elongata의 시료에서 그람 음성, 막대형, 비편모, 비활주, 절 대호기성, 옅은 오렌지색 세균인 2012CJ34-3<sup>T</sup> 균주를 분리하 였다. 16S rRNA 유전자 서열 분석에 기초하여, 2012CJ34-3<sup>T</sup> 균주는 Gaetbulibacter marinus KCTC 23081<sup>T</sup> (96.19%), Gaetbulibacter lutimaris KCTC 23716<sup>T</sup> (95.76%), Jejuia marina KCTC 42342<sup>T</sup> (95.70%), *Gaetbulibacter aquiaggeris* KCTC 42198<sup>T</sup> (94.99%)와 밀접한 관련이 있는 것으로 나타났다. 이 균주는 Gaetbulibacter 종들과 16S rRNA 유전자 유사도가 더 높게 나타났으나, 계통분석 결과 Jejuia 속에 속하는 것으로 나 타났다. 균주 2012CJ34-3<sup>T</sup>와 Jejuia pallidilutea DSM 21165<sup>T</sup> 사이의 평균 뉴클레오타이드 동일성(ANI) 및 디지털 DNA-DNA 혼성화(DDH) 값은 각각 75.0%와 19.20%였다. 규주의 생장은 10~30°C에서 1~10% NaCl (w/v) 및 pH 6.0~8.0의 MA 배지에서 일어난다. 유전체 DNA의 DNA G+C 함량은 31.60 mol%이며, menaquinone-6 (MK-6)이 주요 호흡기 퀴논이다. 주요 세포 지방산(> 5%)은 C15:0 iso, C15:1 iso G, C15:0 iso 3OH 및 summed feature 3 (C<sub>16:1</sub> *ω*7*c* 및/또는 C<sub>16:1</sub> *ω*6*c*)였다. 극성 지질은 포스파티딜에탄올아민, 미확인 아미노인지질, 미확인 아미노지질, 미확인 당지질 및 두 개의 미확인 지질로 구성되 었다. 생리학적 및 생화학적 특성에 따라 균주 2012CJ34-3<sup>T</sup>를 Jejuia 속의 신종으로, 그 이름으로 Jejuia spongiicola sp. nov. 을 제안한다. 기준 균주는 2012CJ34-3<sup>T</sup> (= KACC 22642<sup>T</sup> = LMG 32583<sup>T</sup>)이다.

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# Conflict of Interest

Jin-Sook Park is Editor of KJM. She was not involved in the review process of this article. Also, Authors have no conflicts of interest to report.

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