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# *Gaetbulibacter jejuensis* sp. nov., Isolated from Seawater

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**A novel marine bacterium, designated strain CNURIC014$^T$ was isolated from coastal seawater of Jeju Island in Korea. Strain CNURIC014$^T$ formed yellow colonies on marine agar 2216 and the cells were Gram-negative, non-motile, strictly aerobic, rod-shaped. The temperature, pH and NaCl ranges for growth were 15-37°C, pH 6.0-9.0 and 1.0-7.0% NaCl. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain CNURIC014$^T$ was most closely related to *Gaetbulibacter marinus* and *Gaetbulibacter saemankumensis*, with a sequence similarity of 95.1% and 94.6%, respectively. The DNA G+C content of the strain was 33.1 mol% and the major respiratory quinone was menaquinone-6. The major cellular fatty acids were iso-$C_{15:1}$ (22.8%), iso-$C_{15:0}$ (18.8%), summed feature 3 (iso-$C_{15:0}$ 2-OH/$C_{16:1}$ $\omega$7*c*, 12.9%) and iso-$C_{17:0}$ 3-OH (11.5%). On the basis of phenotypic, phylogenetic, and genotypic data, strain CNURIC014$^T$ represents a novel species within the genus *Geatbulibacter*, for which the name *Gaetbulibacter jejuensis* sp. nov. is proposed. The type strain is CNURIC014$^T$ (=KCTC 22615$^T$ =JCM 15976$^T$).**

***Keywords*:** *G. jejuensis* sp. nov., *Flavobacteriaceae*, taxonomy, seawater

The genus *Gaetbulibacter* belongs to the family *Flavobacteriaceae* (Reichenbach, 1989; Bernardet *et al.*, 2002) of the phylum *Bacteroidetes* was first described by Jung *et al.* (2005). This genus currently contains two recognized species (http://www.bacterio.cict.fr/f/formosa.html): *Gaetbulibacter saemankumensis* from a tidal flat sediment (Jung *et al*., 2005) and *Gaetbulibacter marinus* from surface seawater (Yang and Cho, 2008). The genus *Gaetbulibacter* is Gram-negative, obligately aerobic or facultatively anaerobic, rod-shaped, yellow pigmented bacteria, and oxidase activity, and gliding motility are species-dependent. This aim of the present study was to elucidate the taxonomic status of strain CNURIC014$^T$ which was isolated from coastal seawater of Jeju island in Korea using polyphasic approach. Based on the results of the present study it is proposed that strain CNURIC014$^T$ represents a novel species within the genus *Gaetbulibacter*.

## Materials and Methods

### Isolation and culture of bacteria strain

The novel strain CNURIC014$^T$ was isolated from seawater sample collected from the coast of Jeju Island in Korea by using a standard serial dilution plating method and incubation on Marine Agar 2216 (MA; Difco, USA) at 25°C for 5 days. Subcultivation was routinely performed on MA at 30°C for 3 days under aerobic condition and the strain was stored at -80°C in Marine Broth (MB, Difco) supplemented with 20% glycerol. This strain was deposited in the Korean Collection for Type Cultures (=KCTC 22615$^T$) and the Japan Collection of Microorganisms (=JCM 15976$^T$)

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### Phenotypic and biochemical characteristics

Colony morphology, size, and colour were examined from cultures grown aerobically on MA at 30°C for 3 days. The cell morphology and size were examined by light microscope (Nikon, FDX-35) and transmission electron microscope (JEM-1010; JEOL, Japan) using cells from exponentially growing cultures (Bernardet *et al*., 2002; Jeon *et al*., 2004). Growth temperature range and optimum were measured at 5 to 45°C (at 5°C intervals) on MA. The pH range and optimum for growth were examined at pH 4.0-11.0 (at 0.5 pH unit intervals). The pH was adjusted with 1 M HCl or 1 M NaOH after sterilization. NaCl requirement and tolerance were determined in the presence of 0-10% (w/v) NaCl (at 1% intervals) in synthetic Zobell agar medium (ZoBell, 1941; 5 g Bacto peptone, 1 g yeast extract, 0.1 g ferric citrate, and 15 g Bacto agar in 1 L DW) prepared with modified artificial seawater [0-10% (w/v) NaCl, 5.94 g $MgSO_4 \cdot 7H_2O$, 4.53 g $MgCl_2 \cdot 6H2O$, 0.64 g KCl, and 1.3 g $CaCl_2$ per L]. Flagellar motility was examined by using wet mounts made from fresh culture grown in 30°C for 2 days, according to the method described by Bowman (2000). Gliding motility was also examined by the hanging drop method described by Bowman (2000). Gram reaction was determined by using the bio-Mérieux Gram Stain kit according to the manufacturer's instructions. Catalase activity was evaluated by the production of oxygen bubbles in 3% (v/v) $H_2O_2$ and oxidase activity was tested by oxidation of 1% (w/v) *N*,*N*,*N'*,*N'*-tetramethyl-*p*-phenyl-enediamine solution. The production of flexirubin type pigments was investigated using the 20% KOH test following the minimal standards for the description of new taxa in the family *Flavobacteriaceae* (Reichenbach, 1989; Bernardet *et al*., 2002). Cellular pigments were extracted from cells grown without light in MB using acetone:methanol (1:1, v/v) as described by Yoon *et al.*

(2008). The cellular residues were removed by centrifugation. An adsorption spectrum (200-800 nm) was obtained from the crude cell extract using a Beckman coulter DU 800 UV-visible spectrophotometer (Varian, USA). Anaerobic growth was tested on MA under anaerobic contidition using the GasPak anaerobic system (BBL, USA) at 30°C for 20 days. The following tests were performed in parallel on strain CNURIC 014$^T$. Hydrolysis of Tween 20, Tween 40, Tween 60, Tween 80, DNA, casein, starch, and carboxymethyl (CM) cellulose was investigated on Marine 2216 agar after 7-day incubation at 30°C according to previously described methods (Lanyi, 1987; Tindall *et al.*, 2007). Other phenotypic and enzymatic characterizations of strain CNURIC014$^T$ were conducted using API 20E, API 20NE, API50CH, and API ZYM test kits (bioMérieux, France). The utilization of different carbon sources was tested with the GN2 MicroPlate (Biolog) according to the manufacturer's instructions. Antibiotic sensitivity was tested by spreading bacterial suspension prepared from artificial sea water or 2% sea salts on MA and applying discs impregnated with the following antibiotics: ampicillin (10 μg), carbenicillin (100 μg), erythromycin (15 μg), gentamycin (10 μg), kanamycin (30 μg), lincomycin (15 μg), neomycin (30 μg), nalidixic acid (30 μg), novobiocin (5 μg), oleandomycin (15 μg), penicillin (10 IU), polymyxin B (300 μg), and tetracycline (30 μg).

### Isoprenoid quinines and cellular fatty acids

The whole-cell fatty acid compositions of strain CNURIC014$^T$ was analyzed according to the instructions of the Microbial Identification System (MIDI; Microbial ID, Inc.) using cells grown on MA at 30°C for 3 days. The major respiratory quinones were analysed by the Korean Culture Center of Microorganisms (KCCM; Seoul, South Korea), using reversed-phased HPLC (Komagata and Suzuki, 1987).

### Determination of G+C content

The G+C content of the DNA was determined by thermal denaturation method (Marmur and Doty, 1962) using Ultrospec 2100 spectrophotometer (Pharmacia Biotech, USA). DNA from *Escherichia coli* K-12 was used as a control.

### Determination of 16S rRNA gene sequence and phylogenetic analysis

Genomic DNA was extracted and purified by using a commercial genomic DNA Extraction kit (Promega, USA), and nearly complete 16S rRNA gene sequence was amplified using the bacterial universal primers (Weisburg *et al.*, 1991). 16S rRNA gene sequence of strain CNURIC 014$^T$ was carried out as described previously (Lane, 1991). The resulting 16S rRNA gene sequence was compared with available 16S rRNA gene sequences in the BLAST program (http://www.ncbi.nlm.nih.gov/blast/) to determine the approximate phylogenetic affiliation and also closest neighbors were aligned using CLUSTAL W software (Thompson *et al*., 1994). Sequence similarity values were computed using Similarity Matrix version 1.1 (Ribosomal Database Project II; http://rdp.cme.msu.edu/index.jsp; Cole *et al*., 2003) and EzTaxon server (http://www.eztaxon.org/; Chun *et al*., 2007). Gaps at the 5′ and 3′ ends of the alignment were omitted for further analyses. Phylogenetic trees were constructed by using the neighbour-joining method (Saitou and Nei, 1987) and maximum parsimony method using the Mega 4.0 program (Tamura *et al*., 2007), with bootstrap values based on 1,000 replications (Felsenstein, 1985). Evolutionary distance matrices were calculated according to the algorithm of the Kimura two-parameter model (Kimura, 1983).

### Nucleotide sequence accession number

The GenBank/EMBL/DDBJ accession number for 16S rRNA gene sequence of strain CNURIC014$^T$ is FJ490367.

## Results and Discussion

### Phenotypic and biochemical characteristics

Cells of strain CNURIC 014$^T$ were Gram-negative, non-motile, and rod-shaped. The colonies grown on Marine 2216 agar medium for 3 days at 30°C were circular, convex, smooth, entire, yellow in colour, and 0.5-3.0 mm in diameter. The strain was able to grow at 15-37°C (optimum 30°C). pH and NaCl ranges for growth are pH 6.0-9.0 (optimum, pH 7.0-8.0) and 1.0-7.0% NaCl (optimum, 2-3% NaCl), respectively. Cells produce non-diffusible carotenoid-type pigments with absorption maximum at 453 nm and 447 nm but no flexirubin-type pigments. The strain was oxidase-positive and catalase-positive but negative for nitrate reduction. Casein, starch, and Tween 80 are hydrolysed but Tween 20 and chitin are not. Additional description of the strain is given in the species description, and Table 1 shows a comparison between the characteristics of CNURIC014$^T$ and two *Gaetbulibacter* species.

### G+C content

The G+C content of strain CNURIC014$^T$ was 33.1 mol%, which is slightly lower than values reported to *G. marinus* (38.1 mol%) and *G. saemankumensis* (34.7-34.9 mol%) (Jung *et al.*, 2005; Yang and Cho, 2008)

### Quinones and cellular fatty acid

The major cellular fatty acids of strain CNURIC014$^T$ were iso-$C_{15:1}$ (22.8%), iso-$C_{15:0}$ (18.8%), Summed feature 3 ($C_{16:1}$ $\omega$7c and/or iso-$C_{15:0}$ 2-OH, 12.9%), iso-$C_{17:0}$ 3-OH (11.5%), $C_{15:0}$ (8.6%), iso-$C_{15:0}$ 3-OH (7.1%), and iso-$C_{16:0}$ 3-OH (5.6%). This fatty acid profile was similar to those of *Gaetbulibacter* species, although there were differences in the presence and proportions of some fatty acids (Table 2). However, this difference may be due to different growth and analysis conditions. The detailed fatty acid compositions of the three strains are compared in Table 2. The major respiratory lipoquinone was menaquinone-6 (MK-6). These chemotaxonomic data are in accordance with data obtained for two *Gaetbulibacter* species (Jung *et al.*, 2005; Yang and Cho, 2008).

### Phylogenetic analysis

Based on 16S rRNA gene sequence (1,404 bp) of strain CNURIC014$^T$ was most closely related to the genus *Gaetbulibacter* in family *Flavobacteriaceae*. The strain was most closely related *G. marinus* (95.1%) and *G. saemankumensis* (94.6%). These values are lower than the theoretical threshold (97%) for the delineation of bacterial species based on 16S rRNA gene sequence similarity (Stackebrandt and Goebel, 1994). In the phylogenetic trees generated in the present study (Fig. 1), strain CNURIC014$^T$ formed a monophyletic clade with genus *Gaetbulibacter.* Although the levels of bootstrap support for the clade were relatively low (<50% in the neighbour-joining tree), the clade was clearly separated from other genera in the family *Flavobacteriaceae*. Also, the topology of the phylogenetic tree generated using a maximum-parsimony algorithm were similar to that of the tree constructed using the neighbour-

**Table 1.** Characteristics that differentiate strain CNURIC014$^T$ from the two other recognized species of the genus *Gaetbulibacter*
Strains: 1, strain CNURIC014$^T$ (data from this study); 2, *G. marinus* IMCC1914$^T$ (data from Yang and Cho, 2008); 3, *G. saemankumensis* (data from Jung *et al*., 2005). +, positive; -, negative; w, weakly positive; NA, not available.

| Characteristic | 1 | 2 | 3 |
|---|---|---|---|
| Cell size (μm) | 0.3-0.5× 1.3-1.8 | 0.6-0.7× 0.8-1.9 | 0.4-0.5× 3.0-4.5 |
| Oxidase activity | + | - | + |
| Nitrate reduction | - | - | + |
| Gliding motility | - | - | + |
| Growth range | | | |
| Temperature (°C) | 15-37 | 3-37 | 13-40 |
| pH | 6-9 | 8-11 | 5.5-8.0 |
| NaCl (%) | 1-7 | 0.5-4.0 | 1-7 |
| Facultative anaerobic growth | - | - | + |
| Hydrolysis of | | | |
| Gelatin | + | + | - |
| Casein | + | + | - |
| Cellulose | + | - | NA |
| Starch | + | - | + |
| Tween 20 | - | NA | + |
| Tween 80 | + | NA | - |
| Enzyme activities | | | |
| Lipase (C14) | + | - | + |
| Trypsine | + | + | - |
| α-Chymotrypsin | + | + | - |
| Naphtol-AS-BI-β-phosphohydrolase | - | + | - |
| α-Glucosidase | - | - | + |
| β-Glucosidase | - | w | - |
| N-Acetyl-β-glucosaminidase | - | - | + |
| Acid production of | | | |
| D-Galactose | - | NA | + |
| Mannitol | + | NA | - |
| D-Glucose | - | NA | + |
| Sucrose | - | NA | + |
| Utilization of | | | |
| D-Galactose | + | - | + |
| D-Melibiose | + | - | - |
| D-Raffinose | + | - | - |
| L-Rhamnose | - | - | + |
| Sucrose | - | + | + |
| D-Trehalose | + | + | - |
| Propionic acid | + | + | - |
| L-Alanine | + | + | - |
| L-Asparagine | + | + | - |
| L-Serine | + | + | - |
| Major menaquinone | MK-6 | NA | MK-6 |
| DNA G+C content (mol%) | 33.1 | 38.1 | 34.7-34.9 |

joining method (data not shown). These data suggested that strain CNURIC014$^T$ represented a novel species within the genus *Gaetbulibacter*.

**Table 2.** Cellular fatty acid content (%) of strain CNURIC014$^T$ and the species of the genus *Gaetbulibacter*
Srains: 1, strain CNURIC014$^T$ (data from this study); 2, *G. marinus* IMCC1914$^T$ (data from Yang and Cho, 2008); 3, *G. saemankumensis* (data from Jung *et al*., 2005). Only those fatty acid amounting to less than 0.5% in all species are not shown. Tr, trace amount (<0.5%); -, not detected; ECL, equivalent chain length.

| Fatty acid | 1 | 2 | 3 |
|---|---|---|---|
| Saturated acids | | | |
| $C_{15:0}$ | 8.6 | 3.9 | 1.5 |
| $C_{16:0}$ | 1.6 | 1.2 | 1.3 |
| Branched saturated acids | | | |
| iso-$C_{13:0}$ | 0.5 | 0.6 | 2.0 |
| iso-$C_{14:0}$ | 0.9 | 1.0 | - |
| iso-$C_{15:0}$ | 18.8 | 20.6 | 23.0 |
| iso-$C_{16:0}$ | 0.9 | 1.2 | - |
| anteiso-$C_{15:0}$ | 0.8 | 4.0 | 10.8 |
| Monounsaturated acids | | | |
| $C_{17:1}$ $\omega$6c | tr | tr | 1.0 |
| Branched monounsaturated acids | | | |
| iso-$C_{15:1}$ | 22.8 | 32.1 | 12.5 |
| anteiso-$C_{15:1}$ | - | 3.4 | 1.5 |
| iso-$C_{17:1}$ $\omega$9c | - | tr | 2.1 |
| anteiso-$C_{17:1}$ $\omega$9c | - | - | 1.3 |
| Hydroxy acids | | | |
| $C_{15:0}$ 2-OH | 1.4 | 0.6 | 1.6 |
| $C_{17:0}$ 2-OH | tr | 1.0 | 2.4 |
| $C_{16:0}$ 3-OH | 0.9 | 1.2 | - |
| iso-$C_{15:0}$ 3-OH | 7.1 | 4.8 | 8.2 |
| iso-$C_{16:0}$ 3-OH | 5.6 | 5.5 | 2.0 |
| iso-$C_{17:0}$ 3-OH | 11.5 | 7.8 | 15.2 |
| Summed feature 3[a] | 12.9 | 4.5 | 10.4 |
| Unknown ECL 11.543 | 1.0 | - | - |
| Unknown ECL 13.565 | 3.7 | - | - |

[a] Summed features represent groups of two or three fatty acids which could not be separated by GLC with the MIDI system. Summed feature 3 comprises $C_{16:1}$ $\omega$7c and/or iso-$C_{15:0}$ 2-OH.

In conclusion, on the basis of phylogenetic analysis and the relative low sequences similarity of 16S rRNA gene to *Gaetbulibacter* speices, combined with differential phenotypic characteristics (Table 1). Strain CNURIC014$^T$ could be classified as a novel species of the genus *Gaetbulibacter*, for which the name *Gaetbulibacter jejuensis* sp. nov. is proposed.

## Description of *Gaetbulibacter jejuensis* sp. nov.

*Gaetbulibacter jejuensis* (je.ju.en'sis. N.L. fem. adj. jejuensis of pertaining to Jeju island in the South Korea, where the type strain was isolated).

Cells are Gram-negative, non-motile, strictly aerobic, and rod-shaped bacterium. After 3 days incubation, colonies on MA are circular, convex, entire, smooth, yellow-coloured, and 0.5-3.0 mm in diameter. Flexirubin-type pigments are absent. The yellow pigments (absorption peaks at 453 and 479 nm) are carotenoid type. Growth occurs at 15.0-37.0°C (optimum, 30°C), at pH 6.0-9.0 (optimum, pH 7.0-8.0), and with 1.0-7.0% NaCl (optimum, 2.0-3.0%). Catalase and oxidase are positive.

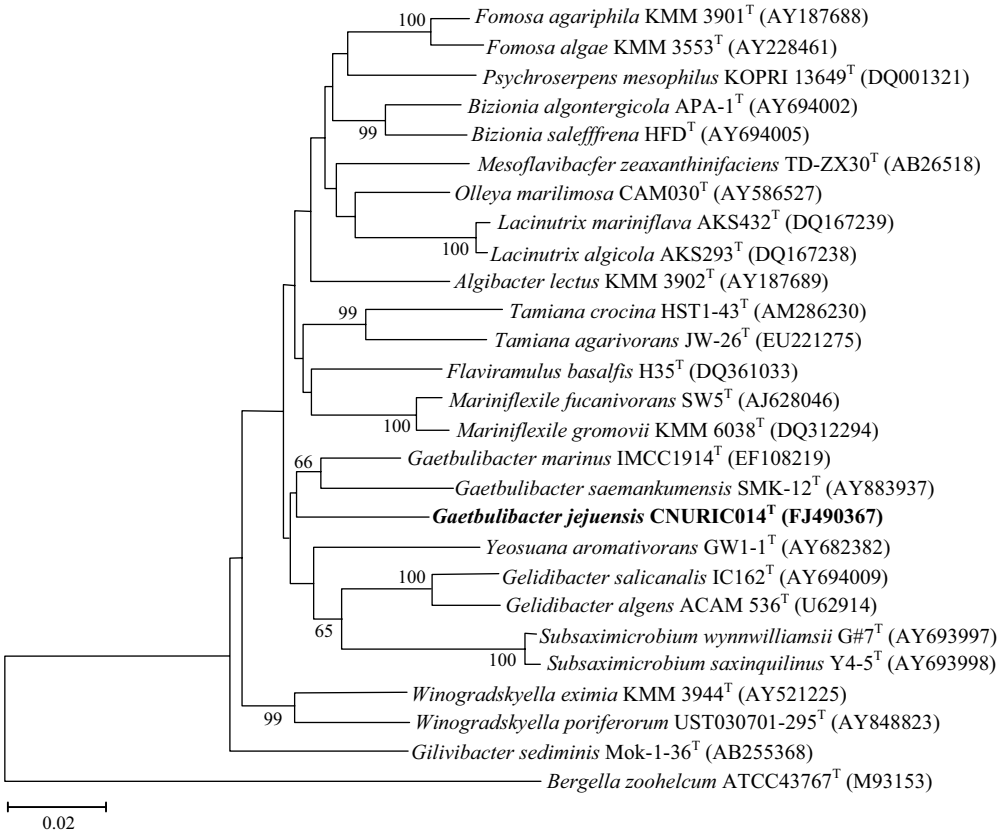


**Fig. 1.** Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequence, showing relationships between strain CNURIC014$^T$ and representative of the family *Flavobacteriaceae*. Bootstrap values are shown in percentages of 1,000 replicates, when greater than 50%. *Bergella zoohelcum* ATCC 43767$^T$ (accession number M93153) was used as an outgroup. Bar=0.02 changes per nucleotide position.

Casein, starch, CM-cellulose, DNA, Tween 40, Tween 60, and Tween 80 are hydrolysed, but Tween 20 and chitin are not. In API 20E tests, production of arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, H2S, and tryptophan deaminase, utilization of citrate, and Voges-Proskauer reaction are negative. In API 20NE tests, Nitrate reduction, indole production, urease activity, arginine dihydrolase activity, glucose fermentation, and gelatin hydrolysis are negative. In the API ZYM tests, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, lipase (C14), and acid phosphatase activities are present. α-Galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase activities are absent. In API 50CH tests, acid is produced from fructose, mannose, mannitol, amygdalin, esculin, cellobiose, maltose, lactose, starch, and glycogen. Acid is not produced from glycerol, erythritol, D-arabinose, L-arabinose, D-Ribose, D-xylose, adonitol, β-Methyl-D-xylose, galactose, glucose, sorbose, rhamnose, dulcitol, sorbitol, α-methyl-D-mannoside, α-methyl-D-glucoside N-acetyl-glucosamine, arbutin, salicin, cellobiose, lactose, melibiose sucrose, trehalose, inulin, melezitose, raffinose, starch, xylitol, gentiobiose, D-turanose, D,L-xylose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-keto-gluconate, and 5-Keto-gluconate. Susceptible to ampicillin (10 μg), carbenicillin (100 μg), erythromycin (15 μg), lincomycin (15 μg), nalidixic acid (30 μg), oleandomycin (15 μg), penicillin (10 IU), and tetracycline (30 μg), but resistant to gentamycin (10 μg), neomycin (30 μg), novobiocin (5 μg), polymyxin B (300 μg), kanamycin (30 μg), and streptomycin (10 μg). The major cellular fatty acids were iso-C15:1 (22.8%), iso-C15:0 (18.8%), summed feature 3 (iso-C15:0 2-OH/C16:1 *ω7c*, 12.9%), and iso-C17:0 3-OH (11.5%). The DNA G+C content of the strain was 33.1 mol% and the major respiratory quinone was menaquinone-6.

The type strain, CNURIC014$^T$ (=KCTC 22615$^T$ =JCM 15976$^T$), was isolated from seawater in Jeju island South Korea.

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