Porticoccus litoralis gen. nov., sp. nov., a gammaproteobacterium isolated from the Yellow Sea

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A marine bacterium, designated IMCC2115^T, was isolated from coastal seawater (Yellow Sea, Korea) using a high throughput cultivation method based on dilution-to-extinction, and taxonomically investigated. Cells of the strain formed tiny, beige to off-white colonies and were Gram-stain-negative, obligately aerobic, chemoheterotrophic, non-motile cocci. Based on 16S rRNA gene sequence comparisons, the strain was most closely related to the genera *Marinimicrobium* (92.0–92.4 %) and *Microbulbifer* (91.6–92.8 %), but phylogenetic trees showed that the strain formed a distinct phyletic line in the class *Gammaproteobacteria* adjacent to the OM60 and SAR92 clades. The DNA G+C content of the strain was 47.8 mol% and the predominant cellular fatty acids were anteiso-$C_{15:0}$ (67.6 %), anteiso-$C_{17:0}$ (14.4 %) and $C_{16:0}$ (6.9 %). The 16S rRNA gene sequence analyses and phenotypic and chemotaxonomic tests allowed the differentiation of IMCC2115^T from other related genera in the class *Gammaproteobacteria*. Therefore, strain IMCC2115^T (=KCCM 42369^T =NBRC 102686^T) is proposed as the representative of a new genus and species, for which the name *Porticoccus litoralis* gen. nov., sp. nov. is proposed.

The oligotrophic marine gammaproteobacteria group was previously reported from a dilution-to-extinction study from the pelagic and coastal regions of the Pacific Ocean (Cho & Giovannoni, 2004). Five distinct clades of oligotrophic marine gammaproteobacteria, including the OM60, BD1-7, KI89A, OM182 and SAR92 clades, and their designations were made only from 16S rRNA gene sequences of previously uncultured bacteria retrieved from various marine environments (Cho & Giovannoni, 2004). The OM60 clade represented by strain HTCC2080 has been shown to contain aerobic, anoxygenic, phototrophic bacteria (Cho *et al.*, 2007) and was most closely related to *Haliea salexigens* DSM19537^T (Urios *et al.*, 2008) and *Congregibacter litoralis* KT71 (Eilers *et al.*, 2001). The BD1-7 clade containing strain HTCC2143 was most closely related to *Dasania marina* KOPRI 20902^T (Lee *et al.*, 2007), forming a sister group to *Spongiibacter marinus* HAL40b^T (Graeber *et al.*, 2008). The SAR92 clade included the proteorhodopsin-containing gammaproteobacterium HTCC2207 (Stingl *et al.*, 2007) and was most closely related to the genus *Microbulbifer* (Gonzalez *et al.*, 1997; Miyazaki *et al.*, 2008; Tanaka *et al.*, 2003; Yoon *et al.*, 2003a, b, 2004, 2007), but is quite different (9 % dissimilarity) in 16S rRNA gene sequence comparisons (Stingl *et al.*, 2007). This study was performed to describe a marine bacterium designated strain IMC2115^T that was phylogenetically closest to the genus *Microbulbifer* and the SAR92 clade on the basis of its 16S rRNA gene sequence. On the basis of phylogenetic analysis and phenotypic characterization, a novel genus and species named *Porticoccus litoralis* gen. nov., sp. nov. is proposed for strain IMCC2115^T and is distinct from other members of the class *Gammaproteobacteria*.

A sample of coastal seawater was collected, at a depth of 1 m, off the Port of Incheon (37° 19′ N 126° 33′ E), Yellow Sea. Strain IMCC2115^T was isolated by using a dilution-to-extinction culturing method in a low nutrient heterotrophic medium that was prepared with 0.2 μm-filtered and autoclaved seawater (Cho & Giovannoni, 2004), and was successfully subcultured on marine agar 2216 (MA; Difco). After determination of the optimum growth temperature, cultures of IMCC2115^T were maintained routinely on MA at 20 °C.

An almost complete sequence of the 16S rRNA gene (1497 bp) was obtained for strain IMCC2115^T as described previously (Cho & Giovannoni, 2003). Phylogenetic ana-

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain IMCC2155^T is EF468719.

A transmission electron micrograph of cells of strain IMCC2115^T and a table detailing the cellular fatty acid profiles of strain IMCC2115^T and previously reported related taxa are available with the online version of this paper.

lyses, including multiple alignment of 16S rRNA gene sequences, determination of sequence similarity and generation of phylogenetic trees, were performed in the ARB package (Ludwig *et al.*, 2004) and PAUP* (Swofford, 2002), as described previously (Cho & Giovannoni, 2006). Sequence comparisons in the ARB database were also confirmed by using the EzTaxon server (Chun *et al.*, 2007) and the results showed that strain IMCC2115^T was most closely related to *Marinimicrobium koreense* KCTC 12356^T (92.4 %) (Lim *et al.*, 2006) and marine gammaproteobacterium HTCC2207 (92.2 %, cultivated but without a validly published name), a member of the SAR92 clade (Stingl *et al.*, 2007). Based on 16S rRNA gene sequence similarities, other relatives of IMCC2115^T included '*Candidatus* Endobugula glebosa' (92.0 %) (Lim & Haygood, 2004), *Microbulbifer agarilyticus* JAMB A3^T (91.9 %) (Miyazaki *et al.*, 2008), *Dasania marina* KOPRI 20902^T (91.7 %) (Lee *et al.*, 2007), *Cellvibrio japonicus* NCIMB 10462^T (91.7 %) (Humphry *et al.*, 2003), *Microbulbifer hydrolyticus* IRE-31^T (91.6 %) (Gonzalez *et al.*, 1997), *Saccharophagus degradans* 2-40^T (91.5 %) (Gonzalez & Weiner, 2000), *Spongiibacter marinus* HAL40b^T (91.3 %) (Graeber *et al.*, 2008), *Teredinibacter turnerae* T7902^T (91.1 %) (Distel *et al.*, 2002), *Congregibacter litoralis* KT71 (90.1 %) (Eilers *et al.*, 2001), *Haliea salexigens* 3X/A02/235^T (90.1 %) (Urios *et al.*, 2008) and *Simiduia agarivorans* SA1^T (Shieh *et al.*, 2008) (90.0 %).

To clarify the phylogenetic position of strain IMCC2115^T, 1125 unambiguously aligned nucleotide positions were used for phylogenetic analyses. Phylogenetic trees were inferred using the maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Fitch, 1971) and neighbour-joining (Saitou & Nei, 1987) algorithms. The robustness of tree topologies for the maximum-likelihood, maximum-parsimony and neighbour-joining trees were evaluated by using bootstrap analyses (Felsenstein, 1985) based on 100, 1000 and 1000 resamplings of the sequences, respectively. All of the phylogenetic trees generated in this study (Fig. 1) indicated that IMCC2115^T was distinctly grouped with uncultured bacterial clones D53 (Zeng *et al.*, 2005) and ELB16-080 (Glatz *et al.*, 2006). This phylogenetic inference together with the level of 16S rRNA gene sequence similarity between strain IMCC2115^T and other cultured representatives (16S rRNA similarities less than 92.4 %) suggested that the strain should be assigned to a novel genus within the class *Gammaproteobacteria*.

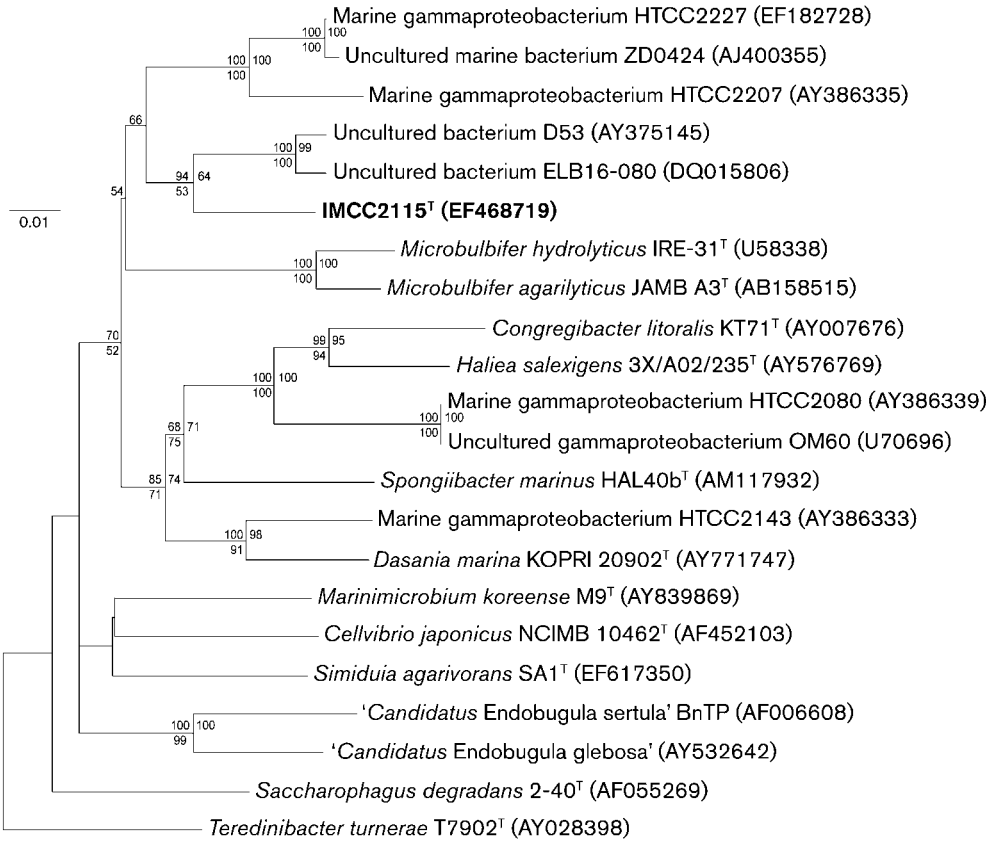

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationships between strain IMCC2115^T and representatives of the related taxa. Bootstrap proportions (above 50 %) are shown from 1000, 1000 and 100 resamplings for neighbour-joining, maximum-parsimony and maximum-likelihood analyses, respectively. *Teredinibacter turnerae* T7902^T (AY028398) was used as an outgroup. Bar, 1 substitution per 100 nucleotide positions.

Cell morphology and size, colony morphology, size and colour, flagella and gliding motility, pigments, and ranges and optima of temperature, pH and salinity for growth were examined using cultures grown on/in MA or marine broth 2216 (MB; Difco) at 20 °C, according to methods described in a previous study (Choi *et al.*, 2007) as well as standard methods (Smibert & Krieg, 1994). Basic biochemical tests and carbon source oxidation tests were performed in API 20NE and API ZYM strips (bioMérieux) and in Biolog GN2 microplates, according to the manufacturers' instructions except that the strips were inoculated with bacterial suspensions in artificial seawater medium (Choo *et al.*, 2007) and incubated at 20 °C for 7 days. Ten different kinds of antimicrobial agents (listed in the species description) were tested by the diffusion plate method (Jorgensen *et al.*, 1999) on MA incubated for 7 days at 20 °C. The DNA G+C content was determined by using HPLC (Mesbah *et al.*, 1989). Cellular fatty acid methyl esters were prepared from cultures grown on MA at 20 °C for 7 days, and also analysed according to the MIDI Microbial Identification System.

Phenotypic characteristics of strain IMCC2115^T were compared with other related species in the class *Gammaproteobacteria* (Table 1) and are summarized in the species description. In short, cells of strain IMCC2115^T were Gram-stain-negative, obligately aerobic, chemoheterotrophic, non-flagellated, non-pigment producing and non-motile cocci (0.38–0.96 μm in diameter, see Supplementary Fig. S1 in IJSEM Online) that form beige to off-white and tiny colonies (0.05 mm in diameter). To check whether IMCC2115^T had light-harvesting systems, such as those carried by the cultured isolates of the OM60 (Cho *et al.*, 2007) or SAR92 (Stingl *et al.*, 2007) clades, PCR primers were used to detect *pufL/pufM* (found in the OM60 clade) and proteorhodopsin (found in the SAR92 clade). Primers pufLf and pufMr were used to amplify the *pufLM* genes for anoxygenic aerobic photoheterotrophy (Béjà *et al.*, 2002; Kim *et al.*, 2007; Nagashima *et al.*, 1997). The following primer sets for proteorhodopsin detection were employed in this study: PRS1f/PRS1r (Sabehi *et al.*, 2005); PRSAR11f/PRSAR11r (Giovannoni *et al.*, 2005); PRfwdf/PRrev (Béjà *et al.*, 2000; Sabehi *et al.*, 2003); and RhodPal-f/RhodPal-r (de la Torre *et al.*, 2003). Conclusively, no genes for photosynthetic reaction centres and proteorhodopsin could be determined for IMCC2115^T using PCR (data not shown). The major fatty acids found in strain IMCC2115^T were anteiso-$C_{15:0}$ (67.6 %), anteiso-$C_{17:0}$ (14.4 %) and $C_{16:0}$ (6.9 %), clearly different from the major fatty acids in other related species (Table 1 and supplementary Table S1).

As shown by the low 16S rRNA gene sequence similarity (<92.2 %) with related species, the distinct phylogenetic relationship (Fig. 1) and several differential phenotypic characteristics (Table 1), strain IMCC2115^T could not be assigned to any known genus. In conclusion, polyphasic evidence collected in this study demonstrates that strain IMCC2115^T belongs to a new species in a new genus of the class *Gammaproteobacteria*, for which the name *Porticoccus litoralis* gen. nov., sp. nov. is proposed.

Description of *Porticoccus* gen. nov.

Porticoccus (Por.ti.coc′cus. L. n. *portus* a harbour, haven, port; N.L. masc. n. *coccus* a coccus; N.L. masc. n. *Porticoccus* a coccus isolated from a harbour).

Gram-stain-negative, chemoheterotrophic, obligately aerobic, oxidase-positive and catalase-negative. Cells are cocci that are devoid of flagella. Requiring NaCl for growth. Visible pigment is not produced. Predominant cellular fatty acids are anteiso-$C_{15:0}$, anteiso-$C_{17:0}$ and $C_{16:0}$. The DNA G+C content of the type strain of the type species is 47.8 mol%. Light-harvesting genes including *pufLM* and proteorhodopsin are not present. Phylogenetically, the genus is affiliated to the class *Gammaproteobacteria*. The type species is *Porticoccus litoralis*.

Description of *Porticoccus litoralis* sp. nov.

Porticoccus litoralis (li.to.ra′lis. L. masc. adj. *litoralis* of or belonging to the sea shore).

Description is the same as that for the genus with the following additional properties. Cells are cocci, 0.38–0.96 μm (typically 0.6 μm) in diameter and are devoid of flagellum and gliding motility. Colonies on MA (grown at 20 °C for 7 days) are circular, convex, entire and opaque. Colonies are beige to off-white and 0.05 mm in diameter. Temperature range for growth is 15–42 °C, optimum 20–25 °C. Growth occurs at pH 5–11 (optimally at pH 7–8) and 1.5–5.0 % NaCl on MA (optimally 3.5 % NaCl). In API 20NE strips, arginine dihydrolase, urease and PNPG (β-galactosidase) are negative. Indole production, hydrolysis of aesculin and gelatin, glucose fermentation and nitrate reduction are absent. In the API ZYM test, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase and acid phosphatase are positive. Alkaline phosphatase, lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are negative. For carbon source utilization (Biolog GN2 microplate), methylamine, glycerol, D-ribose, D-fructose, D-mannose, D-maltose, D-xylitol and L-lysine are positive; methanol, ethanol, D,L-glyceraldehyde, D-arabinose, D-galactose, *N*-acetyl-D-glucosamine, D-glucosamine, α-D-glucose, rhamnose, cellobiose, lactose, melibiose, D-sucrose, D-trehalose, D-melezitose, D-raffinose, adonitol, arabitol, D-mannitol, *myo*-inositol, D-sorbitol, citric acid, gluconic acid, glucuronic acid, pyruvic acid, L-alanine, L-arginine, glycine, L-histidine, L-ornithine, L-proline and L-serine are weakly positive. Carbon source tests for itaconic acid, malonic acid, propionic acid, succinic acid, L-glutamic acid and L-leucine are negative. Susceptible to erythromycin (15 μg) and rifampicin (50 μg). Resistant to ampicillin (10 μg), chloramphenicol (25 μg), gentamicin (10 μg), kanamycin (30 μg), penicillin G (10 μg), streptomycin (10 μg), tetracycline (30 μg) and vancomycin (30 μg). In cultures

Table 1. Characteristics that differentiate strain IMCC2115^T from other genera

Strains: 1, IMCC2115^T (data from this study); 2, *Cellvibrio japonicus* NCIMB 10462^T (Humphry *et al.*, 2003); 3, *Dasania marina* KOPRI 20902^T (Lee *et al.*, 2007); 4, *Haliea salexigens* 3X/A02/235^T (Urios *et al.*, 2008); 5, *Marinimicrobium koreense* KCTC 12356^T (Lim *et al.*, 2006); 6, *Microbulbifer agarilyticus* JAMB A3^T (Miyazaki *et al.*, 2008); 7, *Microbulbifer hydrolyticus* IRE-31^T (Gonzalez *et al.*, 1997); 8, *Saccharophagus degradans* 2-40^T (Gonzalez & Weiner, 2000); 9, *Simiduia agarivorans* SA1^T (Shieh *et al.*, 2008); 10, *Spongiibacter marinus* HAL40b^T (Graeber *et al.*, 2008). +, Positive; −, negative; (+), weakly positive; ND, no data available. Data for strains 2–10 were compiled from previous studies.

Characteristic	1	2	3	4	5	6	7	8	9	10
Cell shape	Cocci	Rods	Irregular rods	Rods	Short rods	Rods	Straight rods	Pleiotrophic	Straight to curved rods	Rods
Flagellation	−	Motile by two polar flagella	Motile by polar flagella	Single polar flagellum	Single flagellum	ND	−	−	Motile with monotrichous flagella (<1 %)	Motile by polar flagellum
Optimal pH for growth	7–8	ND	7.0–8.0	8	7.0–7.5	7.5–8.0	7.5	7.5	–	7–9
pH range for growth	5–11	6–9	7.0–10.0	5.0–9.0	6.0–10.5	6.5–9.5	6.5–8.5	4.5–10.0	7–10	6.5–9.5
Salinity range for growth (% w/v)	1.5–5.0	2	1–9	0.7–7	0–15	7	0.58–5.8	10	0.5–7	1–7
Optimal salinity range (% w/v)	3.5	ND	3–4	4.2	1–3	2–3	0.58–2.9	2.3–3.5	2–3	3
Optimal growth temperature (°C)	20–25	ND	17–22	25–30	35–40	31–35	37	ND	30–35	20–30
Temperature range for growth (°C)	15–42	17–47	4–30	10–37	10–45	10–38	10–41	4–37	15–40	10–40
Nitrate reduction to nitrite	−	+	+	ND	−	+	−	−	+	ND
Oxidase	+	+	+	+	−	+	+	+	+	+
Catalase	−	+	+	+	−	+	+	+	+	(+)
Hydrolysis of:										
Aesculin	−	+	−	ND	+	−	ND	ND	+	ND
Agar	−	−	ND	ND	−	+	−	+	+	ND
Gelatin	−	−	−	ND	−	+	+	+	+	ND
DNA G+C content (mol%)	47.8	48.8	37	61	57	55.2	57.6	46	55.6	69.1
Major fatty acids (% of total)	ai-$C_{15:0}$ (67.6), ai-$C_{17:0}$ (14.4)	$C_{16:1}$ (42.5), $C_{18:1}$ (24.6)	$C_{16:1}\omega7c$+ i-$C_{16:0}$ 2-OH (45.3), $C_{16:0}$ (18.4)	$C_{17:1}\omega8c$ (23.9), $C_{16:1}\omega7c$+ i-$C_{15:0}$ 2-OH (21.2)	$C_{16:0}$ (24.5), $C_{19:1}$ cyclo $\omega8c$ (22.4)	$C_{18:1}$ (27.0), i-$C_{15:0}$ (21.0)	i-$C_{15:0}$ (26.2), i-$C_{17:1}$ $\omega9c$ (25.4)	$C_{16:0}$ (37.0), $C_{14:0}$ (15.0)	$C_{16:1}\omega7c$+ i-$C_{15:0}$ 2-OH (28.6), $C_{17:1}\omega8c$ (22.8)	$C_{17:1}\omega8c$ (51.7), $C_{17:0}$ (9.6)

grown on MA at 20 °C for 7 days, the major cellular fatty acids are anteiso-$C_{15:0}$, anteiso-$C_{17:0}$, $C_{16:0}$, iso-$C_{17:0}$, iso-$C_{15:0}$ and anteiso-$C_{19:0}$ (Table 1 and Supplementary Table S1).

The type strain, IMCC2115^T (=KCCM 42369^T =NBRC 102686^T), was isolated from coastal seawater of the Yellow Sea in Korea (37° 19′ N 126° 33′ E).

Acknowledgements

This study was supported by the Korea Research Foundation Grant funded by the Korean Government (MEST) (KRF-2007-314-C00265) and by the 21C Frontier Program of Microbial Genomics and Applications from the MEST, Korea.

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