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TAXONOMIC DESCRIPTION

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Colwellia maritima sp. nov. and *Polaribacter marinus* sp. nov., isolated from seawater

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ABSTRACT

Two Gram-stain-negative, catalase- and oxidase-positive, and aerobic bacteria, strains MSW7^T and MSW13^T, were isolated from seawater. Cells of strains MSW7^T and MSW13^T are motile and non-motile rods, respectively. Strain MSW7^T optimally grew at 25 °C and pH 7.0 and in the presence of 3% (w/v) NaCl, whereas strain MSW13^T optimally grew at 25 °C and pH 6.0–7.0 and in the presence of 2% NaCl. As the sole respiratory quinone and the major fatty acids and polar lipids, strain MSW7^T contained ubiquinone-8, $C_{16:0}$, $C_{15:1}\,\omega8c$, $C_{17:1}\,\omega8c$ and summed feature 3 ($C_{16:1}\,\omega7c$ and/or $C_{16:1}\,\omega6c$), and phosphatidylethanolamine and phosphatidylglycerol, respectively, whereas strain MSW13^T contained menaquinone-6, $C_{15:1}\,\omega6c$, iso-$C_{15:0}$, anteiso-$C_{15:0}$, and iso-$C_{15:0}$ 3-OH, and phosphatidylethanolamine, respectively. The DNA G+C contents of strains MSW7^T and MSW13^T were 37.3 and 29.9%, respectively. Phylogenetic analyses based on 16S rRNA gene sequences showed that strains MSW7^T and MSW13^T were most closely related to *Colwellia echini* A3^T and *Polaribacter atrinae* WP25^T with 98.8 and 98.1% sequence similarities, respectively. The average nucleotide identity and digital DNA–DNA hybridization values between strain MSW7^T and *C. echini* A3^T and between strain MSW13^T and *P. atrinae* KACC 17473^T were 73.6 and 22.6% and 80.4 and 23.8%, respectively. Based on phenotypic, chemotaxonomic and phylogenetic data, strains MSW7^T and MSW13^T represent novel species of the genera *Colwellia* and *Polaribacter*, respectively, for which the names *Colwellia maritima* sp. nov. and *Polaribacter marinus* sp. nov. are proposed, respectively. The type strains of *C. maritima* sp. nov. and *P. marinus* sp. nov. are MSW7^T (=KACC 22339^T=JCM 35001^T) and MSW13^T (=KACC 22341^T=JCM 35021^T), respectively.

INTRODUCTION

The genus *Colwellia*, a member of the family *Colwelliaceae* within the phylum *Pseudomonadota*, was first proposed by Deming *et al.* [1] through the reclassification of *Vibrio psychroerythrus* as *Colwellia psychrerythraea*. At the time of writing, the genus includes 22 species with validly published names, which all were isolated from marine environments, including seawater [2, 3], marine sediment [4, 5], and marine organisms [6, 7]. In contrast, the genus *Polaribacter*, a member of the family *Flavobacteriaceae* in the phylum *Bacteroidota*, was first proposed by Gosink *et al.* [8] with the descriptions of *Polaribacter irgensii*, *Polaribacter franzmanii* and *Polaribacter filamentus* and the reclassification of *Flectobacillus glomeratus* as *Polaribacter glomeratus*. Emended descriptions of the genus *Polaribacter* have subsequently been proposed by Fukui *et al.* [9], Kim *et al.* [10] and Li *et al.* [11]. At the time of writing, the genus includes 25 species with validly published names, which have been also isolated from marine-related environments [12, 13] and marine organisms [14, 15]. In this study, two novel bacterial strains of the genera *Colwellia* and *Polaribacter*, strains MSW7^T and MSW13^T, were isolated from a seawater sample and taxonomically characterized using a polyphasic approach.

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Keywords: *Colwellia maritima*; *Polaribacter marinus*; seawater; new taxa; *Pseudomonadota*; *Bacteroidota*.
Abbreviations: ANI, average nucleotide identity; ASW, artificial seawater; DDH, DNA–DNA hybridization; MA, marine agar; MB, marine broth; MK, menaquinone; ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; Q, ubiquinone.
The GenBank accession numbers for the 16S rRNA gene and genome sequences of strains MSW7^T and MSW13^T are MZ310521 and MZ189168 and JAKKSL000000000 and JAKQYM000000000, respectively.
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Three supplementary figures and two supplementary tables are available with the online version of this article.

ISOLATION AND ECOLOGY

A seawater sample was collected from a coastal area of Oido (37° 23′ 42″ N 126° 44′ 19″ E), Gyeonggi Province, Republic of Korea, in March 2021. Two putative novel strains, MSW7^T and MSW13^T, were isolated from the seawater sample, as described previously with some modifications [16]. In brief, the collected seawater sample was serially diluted in artificial seawater (ASW; 20 g NaCl, 2.9 g $MgSO_4$, 4.53 g $MgCl_2{\cdot}6H_2O$, 0.64 g KCl and 1.75 g $CaCl_2{\cdot}2H_2O$ per litre), spread on marine agar (MA; MBcell), and incubated for 5 days at 25 °C under an aerobic condition. The 16S rRNA genes of colonies grown on MA were amplified by PCR using the universal primers F1 (5′-AGAGTTTGATCMTGGCTCAG-3′) and R13 (5′-TACGGYTACCTTGTTACGACT T-3′) and the resulting PCR products were double-digested with *Hae*III and *Hha*I. Representative PCR amplicons showing unique fragment patterns were partially sequenced using the universal primer 340F (5′-CCTACGGGAGGCAGCAG-3′) and the sequences were compared with those of all type strains of validly published species in the EzBioCloud server (www.ezbiocloud.net/identify) [17]. Two putative novel strains, designated as MSW7^T and MSW13^T, were selected for further taxonomic characterizations. Strains MSW7^T and MSW13^T were subsequently purified by repeated streaking on fresh MA and routinely cultured aerobically on MA for 3 days at 25 °C. For a long-term storage, strains MSW7^T and MSW13^T were stored at −80 °C in marine broth (MB; MBcell) containing 15% (v/v) glycerol. *Colwellia echini* LMG 30125^T, *Colwellia psychrerythraea* DSM 8813^T, *Polaribacter atrinae* KCTC 42039^T, *Polaribacter haliotis* KCTC 52418^T and *Polaribacter sejongensis* KCTC 23670^T were obtained from their culture collection centres and used as reference strains for the comparison of phenotypic properties and fatty acid compositions.

16S rRNA GENE PHYLOGENY

The 16S rRNA gene products of strains MSW7^T and MSW13^T amplified by PCR with the primers F1 and R13 were further sequenced using the universal primers 518R (5′-ATTACCGCGGCTGCTGG-3′) and 805F (5′-GATTAGATACCCTGGTAGTC-3′) at Macrogen (Seoul, Republic of Korea). The sequences obtained by 340F, 518R, and 805F primers were assembled and near complete 16S rRNA gene sequences of strains MSW7^T (1479 nucleotides) and MSW13^T (1448 nucleotides) were obtained. The 16S rRNA gene sequences of strains MSW7^T and MSW13^T were compared with the 16S rRNA gene sequences of all available bacterial type strains using the EzBioCloud server (www.ezbiocloud.net) [17]. The 16S rRNA gene sequences of strains MSW7^T and MSW13^T and closely related type strains were aligned using the fast secondary-structure aware Infernal aligner [18]. Phylogenetic trees using the neighbour-joining (NJ), maximum-likelihood (ML) and maximum-parsimony (MP) algorithms with bootstrap values (1000 replications) were reconstructed using MEGA 11 software [19], based on the Kimura two-parameter model, nearest-neighbor-interchange heuristic search method, and complete deletion options, respectively.

Comparative analysis of 16S rRNA gene sequences revealed that strain MSW7^T was most closely related to *C. echini* A3^T and *C. psychrerythraea* ATCC 27364^T with 98.8 and 98.3% sequence similarities, respectively, and strain MSW13^T was most closely related to *P. atrinae* WP25^T and *P. haliotis* RA4-7^T with 98.1 and 97.8% sequence similarities, respectively. Phylogenetic analysis based on the NJ algorithm of the 16S rRNA gene sequences showed that strain MSW7^T formed a phylogenetic lineage with *C. echini* A3^T within the genus *Colwellia* and strain MSW13^T formed a distinct lineage from *P. atrinae* WP25^T and *P. litorisediminis* OITF-11^T within the genus *Polaribacter* (Fig. 1). Phylogenetic trees reconstructed using ML and MP algorithms also showed that strains MSW7^T and MSW13^T were clustered with the members of the genera *Colwellia* and *Polaribacter*, respectively (Fig. S1 available in the online version of this article). Comparative and phylogenetic analyses of 16S rRNA gene sequences suggest that strains MSW7^T and MSW13^T represent novel species of the genera *Colwellia* and *Polaribacter*, respectively.

GENOME FEATURES

For the whole-genome sequencing, the genomic DNAs of strains MSW7^T and MSW13^T were extracted from their cultured cells in MB using a Wizard Genomic DNA purification kit (Promega), according to the manufacturer's instructions. The genomic DNA of strain MSW7^T was sequenced using an Oxford Nanopore MinION sequencer, and the sequencing reads were *de novo*-assembled using Unicycler (version 0.4.9b) [20]. On the other hand, the genomic DNA of strain MSW13^T was sequenced on an Illumina HiSeq X sequencing platform (Macrogen, Seoul, Republic of Korea) with 151 bp paired-end reads. The paired-end sequencing reads were *de novo*-assembled using SPAdes (version 3.11.1) [21]. The quality of the assembled genomes of strains MSW7^T and MSW13^T was evaluated based on their completeness and contamination rates using the CheckM program (version 1.1.3) [22]. The draft genomes of strains MSW7^T and MSW13^T were annotated by the NCBI Prokaryotic Genome Annotation Pipeline [23].

The *de novo* assembly of the genome sequencing data of strains MSW7^T and MSW13^T were resulted in the draft genomes of 5.2 and 3.4 Mb sizes with average genome coverages of approximately 32× and 1212×, respectively. The 16S rRNA gene sequences in the genomes of strains MSW7^T and MSW13^T were identical to those obtained from PCR-based sequencing. The draft genomes of strains MSW7^T and MSW13^T comprised nine and 50 contigs with the N50 values of 1152 and 297 kb, respectively. The genomes of strain MSW7^T and MSW13^T were predicted to have a total of 5201 and 3070 genes, respectively, and among them, 4180 and 3020 protein-coding genes, 83 and 42 RNA genes, and 63 and 35 tRNA genes were predicted, respectively (Table 1). The DNA G+C contents of strains MSW7^T and MSW13^T were calculated as 37.3and 29.9mol%, respectively.

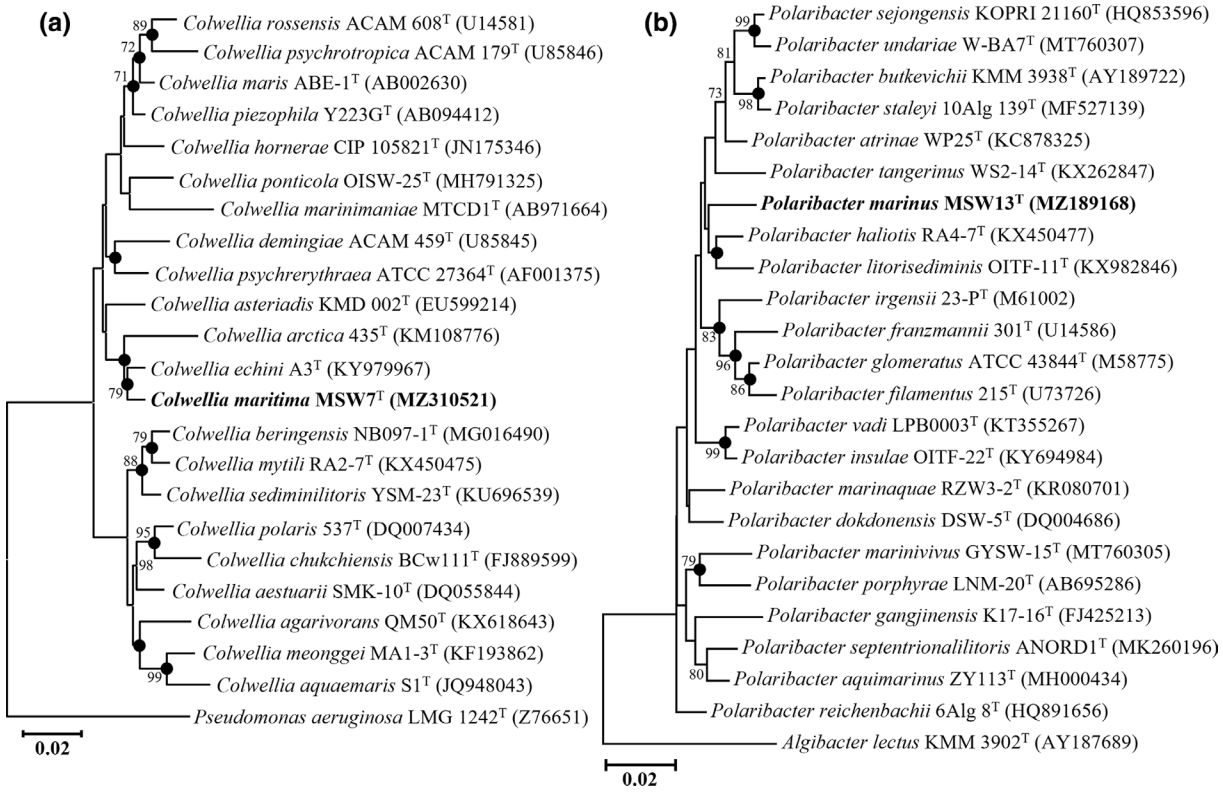

Fig. 1. Neighbour-joining trees showing the phylogenetic relationships between strains MSW7^T (a) and MSW13^T (b) and their closely related *Colwellia* (a) and *Polaribacter* (b) type strains, respectively, based on the 16S rRNA gene sequences. Bootstrap values (>70%) based on 1000 replicates are shown on branch nodes. Closed circles (●) indicate the nodes that were also recovered in the maximum-likelihood and maximum-parsimony trees. *Pseudomonas aeruginosa* LMG 1242^T (Z76651) and *Algibacter lectus* KMM 3902^T (AY187689) were used as the outgroups for the genera *Colwellia* and *Polaribacter*, respectively. The scale bars indicate 0.02 changes per nucleotide position.

For the genome-based phylogenetic analysis, 92 core genes were extracted from the genomes of strains MSW7^T and MSW13^T and closely related type strains, and their amino acid sequences were concatenated and aligned using the up-to-date bacterial core gene pipeline [24]. A phylogenomic ML tree with bootstrap values (1000 replications), based on the concatenated amino acid sequences, was reconstructed using MEGA7 software. Average nucleotide identity (ANI) and digital DNA–DNA hybridization (DDH) values between strains MSW7^T and MSW13^T and their closely related type strains were calculated using OrthoANI [25] and Genome-to-Genome Distance Calculator [26], respectively.

The phylogenomic ML tree based on 92 housekeeping core genes showed that strains MSW7^T and MSW13^T formed distinct lineages within the genera *Colwellia* and *Polaribacter* (Fig. 2), respectively, representing novel members of the genera *Colwellia* and *Polaribacter*, respectively. The ANI and digital DDH values between strain MSW7^T and *C. echini* A3^T, the most closely related type strain, were 73.6 and 22.6%, respectively, and the ANI and digital DDH values between strain MSW13^T and *P. atrinae* KACC 17473^T, the most closely related type strain, were 80.4 and 23.8%, respectively, which were clearly lower than the thresholds (ANI, ~95%; digital DDH, 70%) for prokaryotic species delineation [27]. The phylogenomic and genome relatedness results clearly suggest that strains MSW7^T and MSW13^T represent novel species of the genera *Colwellia* and *Polaribacter*, respectively.

It has been reported that many *Colwellia* and *Polaribacter* species have an ability to degrade algal polysaccharides that are abundant in marine environments [2, 7, 8, 28–30], therefore, the ability to degrade polysaccharides can be an important characteristic of *Colwellia* and *Polaribacter* species. To infer the genetic potential of strains MSW7^T and MSW13^T for carbohydrate degradation, genes encoding carbohydrate-active enzymes (CAZys) in the genomes of strains MSW7^T and MSW13^T and the reference strains were analysed using dbCAN2 meta server [31].

The genomes of strains MSW7^T and MSW13^T were predicted to harbour a total of 70 and 83 CAZy genes, respectively, which were slightly fewer than those in the genomes of the reference strains, *C. echini* A3^T (121), *C. psychrerythraea* 34H^T (91), *P. atrinae* KACC 17473^T (135), *P. haliotis* RA4-7^T (162) and *P. sejongensis* KCTC 23670^T (189) (Table 1), suggesting that strains MSW7^T and MSW13^T have lower metabolic abilities for polysaccharides. Particularly, genes encoding glycoside hydrolase (GH) family 16 containing laminarinase and carrageenase were identified in the genomes of *C. echini* A3^T and *C. psychrerythraea* 34H^T, but not

Table 1. Genome sequencing summary and comparison of general genome features of strains MSW7^T and MSW13^T and their reference strains

Strains: 1, MSW7^T (JAKKSL000000000); 2, *C. echini* A3^T (PJAI00000000); 3, *C. psychrerythraea* 34H^T (CP000083); 4, strain MSW13^T (JAKQYM000000000); 5, *P. atrinae* KACC 17473^T (LVWE00000000); 6, *P. haliotis* RA4-7^T (NIDK00000000); 7, *P. sejongensis* KCTC 23670^T (CP019336).

Characteristics	Taxa						
	1	2	3	4	5	6	7
Genome status*	D	D	C	D	D	D	C
No. of contig	9	146	1	50	113	29	1
Genome size (Mb)	5.2	4.3	5.4	3.4	3.9	3.8	4.5
G+C contents (%)	37.3	36.9	40	29.9	31.2	30.5	30
No. of total genes	5201	3504	4570	3070	3416	3,268†	3668
No. of protein-coding genes	4180	3377	4403	3020	3260	3,226†	3597
No. of total RNA	83	93	120	42	50	42†	42
No. of tRNA	63	78	88	35	42	36†	38
No. of carbohydrate-active enzymes:‡	70	121	91	83	135	162	189
Glycoside hydrolase	37	59	44	25	73	88	107
Glycosyltransferase	18	23	22	38	40	31	46
Polysaccharide lyase	7	12	0	5	3	13	13
Carbohydrate esterase	5	6	11	11	12	17	14
Auxiliary activities	2	5	9	1	1	2	0
Carbohydrate-binding module	1	16	5	3	6	11	9

*D, draft; C, complete.
†The genome of *P. haliotis* RA4-7^T was annotated using Prokka [41] in this study because genome sequence from NCBI Assembly was not annotated.
‡Carbohydrate-active enzyme of all genomes were analysed using dbCAN2 meta server [31] in this study.

in the genome of strain MSW7^T. However, genes encoding polysaccharide lyase (PL) family 29 containing chondroitin-sulphate endolyase were present in strain MSW7^T (three genes), but not in *C. echini* A3^T and *C. psychrerythraea* 34H^T, suggesting that strain MSW7^T may have different range of metabolic capabilities for carbohydrates from its reference strains. Strain MSW13^T has fewer GH family genes that are highly present in other reference strains. For example, GH29, GH30_1, GH95 and GH141 genes containing fucosidase and xylanase were not identified in strain MSW13^T. Several *Polaribacter* strains harbour a light-activated proton pump, proteorhodopsin, in their genomes [32, 33], but BLASTP analyses based on the proteorhodopsin genes of *Polaribacter* sp. MED152 (EAQ40925) and *Polaribacter dokdonensis* DSW-5 (KOY50829) as query sequences showed that strain MSW13^T and its reference strains do not have proteorhodopsin gene homologues.

MORPHOLOGY AND PHYSIOLOGY

The growth of strains MSW7^T and MSW13^T was assessed on various agar media, including MA, Reasoner's 2A (R2A) agar (MBcell), Luria–Bertani (LB) agar (MBcell), tryptic soy agar (MBcell), brain-heart infusion (MBcell) agar, and nutrient agar (MBcell) containing 2.0% (w/v) NaCl. The growth temperature and pH of strains MSW7^T and MSW13^T were tested at different temperatures (4, 10, 15, 20, 25, 30, 37, 40 and 45 °C) and pH values (4.0–11.0, at 1.0 pH intervals) on MA and in MB, respectively. MB media with pH 4.0–5.0, pH 6.0–7.0, pH 8.0–9.0, and pH 10.0–11.0 values were prepared using sodium citrate, Na_2HPO_4/NaH_2PO_4, Tris-HCl, and NaOH buffers, respectively, and their pH values were adjusted after autoclaving (at 121 °C for 15 min) if necessary. Salt tolerance of strains MSW7^T and MSW13^T was tested in MB with different NaCl concentration (0–10% at 1.0% intervals, w/v) prepared in laboratory according to the MB formula. All physiological and biochemical tests of strains MSW7^T and MSW13^T were performed for 3 days under their optimal growth conditions. Cell morphology was investigated using a phase-contrast microscope (Axio Scope.A1, Zeiss) and a transmission electron microscope (JEM-1010, JEOL). Gram staining was conducted using the bioMérieux Gram-stain kit, according to the manufacturer's instructions. Oxidase activity was assessed by the oxidation of 1% (w/v) tetramethyl-*p*-phenylenediamine and catalase activity was determined by the production of oxygen bubbles in 3% (v/v) aqueous hydrogen peroxide solution. Anaerobic growth of strains MSW7^T and MSW13^T was tested on MA and MA

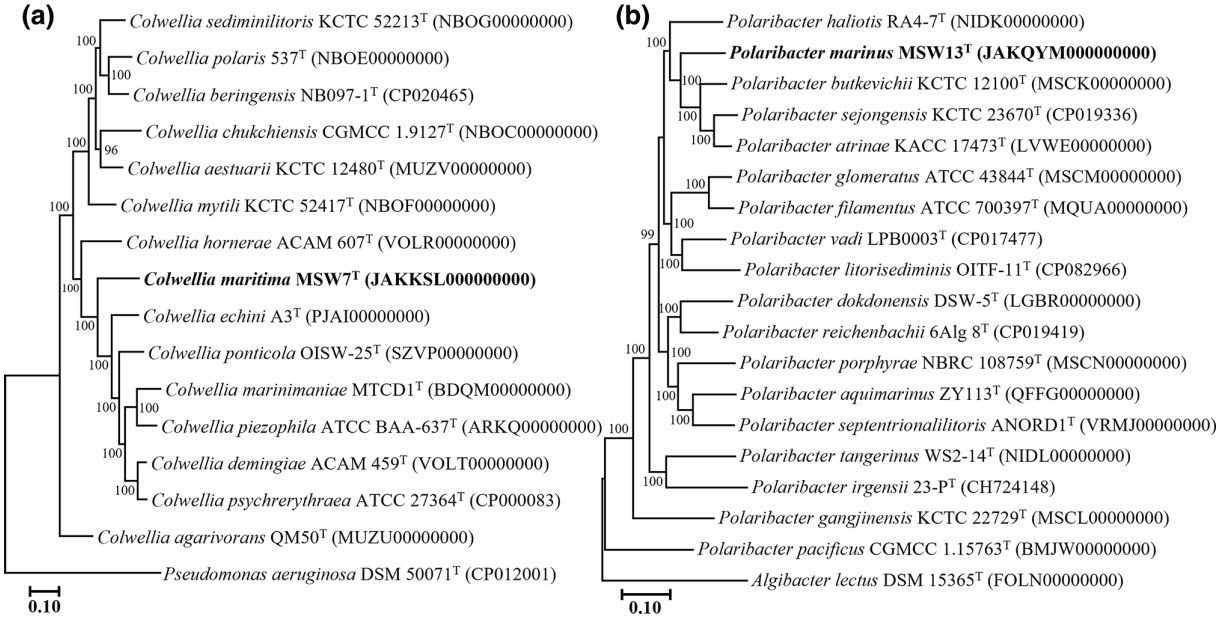

Fig. 2. Maximum-likelihood phylogenomic trees showing the phylogenetic relationships between strains MSW7^T (a) and MSW13^T (b) and their closely related *Colwellia* (a) and *Polaribacter* (b) type strains, respectively, based on the concatenated amino acid sequences of 92 housekeeping core genes. Bootstrap values (>70%) based on 1000 replicates are shown on branch nodes. *Pseudomonas aeruginosa* DSM 50071^T (CP012001) and *Algibacter lectus* DSM 15365^T (FOLN00000000) were used as the outgroups for the genera *Colwellia* (a) and *Polaribacter* (b), respectively. The scale bars indicate 0.1 changes per amino acid.

supplemented with 0.1% (w/v) potassium nitrate under anaerobic conditions using the GasPak Plus system (BBL) for 21 days. The production of flexirubin-type pigments was analysed by adding 20% (w/v) KOH to the cells as described previously [34]. The following phenotypic characteristics of strains MSW7^T and MSW13^T were investigated with the reference strains in parallel under the same conditions at their optimum temperatures. Hydrolysis of casein (1% skimmed milk, w/v), starch (1%), aesculin (0.1%), L-tyrosine (0.5%), Tween 20 (1%) and Tween 80 (1%) was assayed on MA, as described previously [35]. Additional enzymatic activities and biochemical features were evaluated using the API 20NE and API ZYM kits (bioMérieux) according to the manufacturers' instructions, except that inocula were prepared by resuspending cells grown in MB in ASW.

Strains MSW7^T and MSW13^T grew well on MA and slowly grew on R2A agar, but did not grow on LB agar, tryptic soy agar, brain–heart infusion agar. However, strains MSW7^T did not grow on nutrient agar, but strain MSW13^T grew slowly on nutrient agar. Cells of strain MSW7^T were motile rods by means of a single polar flagellum with 0.7–0.8×1.8–2.1 µm in size, whereas cells of strain MSW13^T were non-motile rods with 0.4–0.6×2.0–2.1 µm in size (Fig. S2). Anaerobic growth of strain MSW7^T was only observed on MA supplemented with nitrate, whereas anaerobic growth of strain MSW13^T was observed on MA regardless of nitrate supplementation, but these results suggest that both strains are facultative aerobic. Many phenotypic, physiological, and biochemical characteristics properties were shared between strains MSW7^T and MSW13^T and their closely related reference strains, but there were also many features that distinguished strains MSW7^T and MSW13^T from the reference strains (Tables 2 and 3).

CHEMOTAXONOMY

Isoprenoid quinones of strains MSW7^T and MSW13^T were extracted and analysed using an HPLC (LC-20A, Shimadzu) system equipped with a reversed-phase column (250×4.6 mm; Kromasil, Akzo Nobel) and a diode array detector (SPD-M20A, Shimadzu) as described previously [36]. For the cellular fatty acid analysis, the cells of strains MSW7^T and MSW13^T and the reference strains were cultivated under their optimal growth conditions and harvested at the exponential phase. The cells were saponified, methylated, and extracted according to the MIDI protocol. The fatty acid methyl esters were analysed by gas chromatography (6890, Hewlett Packard) and identified based on RTSBA6 database of the Microbial Identification System (Sherlock version 6.0B) [37]. Polar lipids extracted from the exponential phase cells were analysed by TLC methods according to the previously described procedure [38]. Total polar lipids, aminolipids, phospholipids, and glycolipids were detected by spray reagents of 10% ethanolic molybdophosphoric acid, ninhydrin, Dittmer–Lester and α-naphthol reagents, respectively [39]. The presence or absence of

Table 2. Differential phenotypic characteristics between strain MSW7^T and closely related type strains of *Colwellia* species

Strains: 1, MSW7^T (this study); 2, *C. echini* LMG 30125^T [7]; 3, *C. psychrerythraea* DSM 8813^T [1, 42]. All strains are positive for the following characteristics: nitrate reduction, activity* of oxidase, catalase, urease, alkaline phosphatase, esterase (C4), acid phosphatase, leucine arylamidase and naphthol-AS-BI-phosphohydrolase, and hydrolysis* of aesculin, Tween 20 and Tween 80. All strains are negative for the following characteristics: indole production, glucose fermentation, activity* of lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, α-galactosidase, *β*-glucuronidase, *β*-glucosidase, *N*-acetyl-*β*-glucosaminidase, α-mannosidase, α-fucosidase and arginine dihydrolase, and assimilation* of L-arabinose, D-mannose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. +, Positive; –, negative; W, weakly positive.

Characteristic	1	2	3
Isolation source	Seawater	Sea urchin	Antarctic sea-ice
Colony colour*	Beige	Off-white	Red
Growth range of:			
Temperature (°C, optimum)	4–30 (25)*	4–25 (20)*	0–19 (10)
NaCl (%, optimum)	1–4 (3)*	1–6 (3)*	2–4 (2–3)
pH (optimum)	5–10 (7)*	7–9 (7)*	7–9 (7)
Hydrolysis of:*			
Agar	–	+	–
Casein	+	–	+
Gelatin	+	–	+
Starch	–	+	+
L-Tyrosine	+	–	–
Assimilation of:*			
D-Glucose	–	+	+
D-Mannitol	–	W	–
Maltose	–	–	+
N-Acetyl-glucosamine	–	+	+
Enzyme activity of:*			
β-Galactosidase	–	+	+
Esterase lipase (C8)	W	+	W
Trypsin	–	W	–
α-Chymotrypsin	–	–	W
α-Glucosidase	–	+	–

*Data from this study.

phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) in strains MSW7^T and MSW13^T was verified using their standard polar lipid compounds purchased from Sigma-Aldrich (USA).

The sole respiratory quinones of strains MSW7^T and MSW13^T were ubiquinone-8 (Q-8) and menaquinone-6 (MK-6), respectively, which were consistent with those of the other type strains of *Colwellia* and *Polaribacter* species, respectively [1–15]. The major fatty acids (>10% of the total fatty acids) of strain MSW7^T were $C_{16:0}$, $C_{15:1}$ *ω8c*, $C_{17:1}$ *ω8c* and summed feature 3 (comprising $C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*) (Table S1), whereas those of strain MSW13^T were $C_{15:1}$ *ω6c*, iso-$C_{15:0}$, anteiso-$C_{15:0}$ and iso-$C_{15:0}$ 3-OH (Table S2). The presence of $C_{16:0}$ and summed feature 3 as major fatty acids in strain MSW7^T was common with those in other reference strains, but the presence of $C_{15:1}$ *ω8c* and $C_{17:1}$ *ω8c* as major fatty acids in strain MSW7^T clearly differentiated the strain from other *Colwellia* species (Table S1). The fatty acid profiles of strain MSW13^T and other reference strains were similar; however, the proportions of some fatty acids were different (Table S2). As polar lipids, PE, PG and two unidentified lipids were identified in strain MSW7^T, whereas PE, three unidentified aminolipids and four unidentified lipids were identified in strain MSW13^T (Fig. S3), which were generally consistent with the other type strains of the genera *Colwellia* and *Polaribacter*, respectively [2, 5, 9, 40].

Table 3. Differential phenotypic characteristics between strain MSW13^T and closely related type strains of *Polaribacter* species.

Strains: 1, MSW13^T (this study); 2, *P. atrinae* KCTC 42039^T [14]; 3, *P. haliotis* KCTC 52418^T [40]; 4, *P. sejongensis* KCTC 23670^T [10]. All strains are positive for the following characteristics: activity* of oxidase, catalase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase, and hydrolysis* of aesculin, L-tyrosine and Tween 20. All strains are negative for the following characteristics: indole production, nitrate reduction, flexirubin-type pigment production, casein hydrolysis*, activity* of lipase (C14), α-chymotrypsin, β-glucuronidase, α-glucosidase, α-mannosidase, α-fucosidase, urease and arginine dihydrolase, and assimilation* of L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, *N*-acetyl-glucosamine, maltose, trisodium citrate and phenylacetic acid. +, Positive; –, negative; w, weakly positive.

Characteristic	1	2	3	4
Isolation source	Seawater	Intestine of comb pen shell	Gut of abalone	Antarctic soil
Colony colour on MA*	Yellow	Yellow	Light yellow	Light yellow
Growth range of:*				
Temperature (°C, optimum)	4–30 (25)	4–30 (20)	4–30 (25)	4–37 (25)
NaCl (%, optimum)	1–5 (2)	1–6 (2)	1–5 (2–3)	0.5–5 (3)
pH (optimum)	5–9 (6–7)	5–9 (7)	5–8 (7)	7–8.5 (7.5)
Glucose fermentation*	–	w	–	+
Acetoin production*	–	+	w	+
Hydrolysis of:*				
Gelatin	+	–	–	+
Starch	–	+	+	+
Tween 80	–	+	+	+
Enzyme activity of:*				
β-Galactosidase	–	+	+	+
Trypsin	+	w	w	w
α-Galactosidase	–	+	w	+
β-Glucosidase	–	–	+	+
N-Acetyl-β-glucosaminidase	–	–	w	–

*Data from this study.

TAXONOMIC CONCLUSION

The phylogenetic inference and phenotypic and chemotaxonomic characteristics support that strains MSW7^T and MSW13^T represent novel species of the genera *Colwellia* and *Polaribacter*, respectively, for which the names *Colwellia maritima* sp. nov. and *Polaribacter marinus* sp. nov. are proposed, respectively.

DESCRIPTION OF *COLWELLIA MARITIMA* SP. NOV.

Colwellia maritima (ma.ri'ti.ma. L. fem. adj. *maritima*, of the marine environment, maritime).

Colonies on MA are beige, circular with entire edges, smooth, and slightly convex with a diameter of 0.5–1 mm after 3 days incubation at 25 °C. Cells are Gram-stain-negative, facultative aerobic, non-spore-forming and motile rods by means of a single polar flagellum (approximately 0.7–0.8 µm wide and 1.8–2.1 µm long). Oxidase- and catalase-positive. Growth occurs at 4–30 °C (optimum, 25 °C) and pH 5.0–10.0 (optimum, pH 7.0) and in the presence of 1.0–4.0% (w/v) NaCl (optimum, 3.0 %). Nitrate is reduced. Aesculin, gelatin, casein, L-tyrosine, Tween 20, and Tween 80 are hydrolyzed, but agar and starch are not. Indole production and D-glucose fermentation are negative. Activity of urease, alkaline phosphatase, esterase (C4), esterase lipase (C8; weakly), leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase is positive, but activity of arginine dihydrolase, β-galactosidase (PNPG), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, α-glucosidase, β-glucosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase is negative. Assimilation of D-glucose, L-arabinose, maltose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, adipic acid, capric acid, malic acid, trisodium citrate and phenylacetic acid is

negative. The sole respiratory quinone is Q-8. The major fatty acids (>10 %) are summed feature 3 ($C_{16:1}$ $\omega7c$ and/or $C_{16:1}$ $\omega6c$), $C_{15:1}$ $\omega8c$, $C_{17:1}$ $\omega8c$ and $C_{16:0}$. PE and PG are identified as the major polar lipids. The DNA G+C content of the type strain is 37.3 mol% (calculated from the whole genome sequence).

The type strain, MSW7^T (=KACC 22339^T=JCM 35001^T), was isolated from seawater of Yellow Sea in South Korea.

DESCRIPTION OF *POLARIBACTER MARINUS* SP. NOV.

Polaribacter marinus (ma.ri′nus. L. masc. adj. *marinus*, of the sea, marine).

Colonies on MA are yellow, circular with entire edges, smooth, glistening, and slightly convex with a diameter of 0.5–1 mm after 3 days of incubation at 25 °C. Cells are Gram-stain-negative, facultative aerobic, and non-motile rods (approximately 0.4–0.6 µm wide and 2.0–2.2 µm long). Oxidase- and catalase-positive. Growth occurs at 4–30 °C (optimum, 25 °C) and pH 5.0–9.0 (optimum, pH 6.0–7.0) and in the presence of 1.0–5.0 % (w/v) NaCl (optimum, 2.0 %). Flexirubin-type pigments are not produced. Tween 20, aesculin, gelatin and L-tyrosine are hydrolysed, but casein, starch and Tween 80 are not. Nitrate is not reduced. Indole production and D-glucose fermentation are negative. Positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities, but negative for arginine dihydrolase, urease, β-galactosidase, lipase (C14), α-chymotrypsin, α-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities. Assimilation of D-glucose, L-arabinose, maltose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, adipic acid, capric acid, malic acid, trisodium citrate and phenylacetic acid is negative. The sole isoprenoid quinone is MK-6. The major cellular fatty acids (>10 %) are anteiso-$C_{15:0}$, iso-$C_{15:0}$, $C_{15:1}$ $\omega6c$ and iso-$C_{15:0}$ 3-OH. PE is identified as the major polar lipid. The DNA G+C content of the type strain is 29.9 mol% (calculated from the whole genome sequence).

The type strain is MSW13^T (=KACC 22341^T=JCM 35021^T), which was isolated from seawater of Yellow Sea in South Korea.

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Conflicts of interest
The authors declare that there are no conflicts of interests.

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