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Devosia rhodophyticola sp. nov. and *Devosia algicola* sp. nov., isolated from a marine red alga

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Abstract

Two Gram-stain-negative, obligately aerobic, motile rod bacteria, designated as G2-5^T and G20-9^T, exhibiting catalase- and oxidase-positive activities, were isolated from the phycosphere of a *Chondrus* species, a marine red alga. Strain G2-5^T exhibited optimal growth at 30°C and pH 5.0–6.0 and in the presence of 0.5–1.0% NaCl. In contrast, strain G20-9^T demonstrated optimal growth at 25°C and pH 6.0 and in the presence of 0.5–1.5% NaCl. Both strains contained ubiquinone-10, summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$), $C_{18:0}$ and 11-methyl-$C_{18:1}\omega7c$, and diphosphatidylglycerol and phosphatidylglycerol as the major respiratory isoprenoid quinone, cellular fatty acids and polar lipids, respectively. The genomic DNA G+C contents were 57.2mol% for strain G2-5^T and 57.5mol% for strain G20-9^T. Strains G2-5^T and G20-9^T exhibited 98.2% 16S rRNA gene sequence similarity, along with 82.3% average nucleotide identity (ANI) and 25.0% digital DNA–DNA hybridization (dDDH) values, indicating that they represent different species. Phylogenetic analyses based on both 16S rRNA gene and genome sequences revealed that strains G2-5^T and G20-9^T formed distinct phylogenic lineages within the genus *Devosia*. Strains G2-5^T and G20-9^T were most closely related to *Devosia limi* DSM 17137^T and *Devosia beringensis* S02^T with 97.7 and 96.9% 16S rRNA gene sequence similarities, respectively. The ANI and dDDH values between strains G2-5^T and G20-9^T and other *Devosia* species were lower than 73.9 and 19.2%, respectively, suggesting that they constitute novel species within the genus *Devosia*. Based on their distinct phenotypic, chemotaxonomic, and molecular characteristics, strains G2-5^T and G20-9^T represent two novel species of the genus *Devosia*, for which the names *Devosia rhodophyticola* sp. nov. (G2-5^T=KACC 22601^T=JCM 35404^T) and *Devosia algicola* sp. nov. (G20-9^T=KACC 22650^T=JCM 35405^T) are proposed, respectively.

INTRODUCTION

The genus *Devosia* belonging to the family *Devosiaceae* of the phylum *Pseudomonadota* was initially established through the reclassification of *Pseudomonas riboflavina* as *Devosia riboflavina* [1] and the genus description was emended by Zhang *et al.* [2]. At the time of writing (December 2023), this genus comprises 35 species with validly published names (https://lpsn.dsmz.de/genus/devosia) [3]. The members of the genus *Devosia* have been isolated from various environmental habitats, including alpine glacial cryoconite [2], marine sediments [4–8], horse blood [9], nitrifying inoculum [10], root nodules and soils [11, 12], and medical leeches [13]. Members of the genus *Devosia* are Gram-stain-negative, aerobic, non-endospore-forming, oxidase-positive rod-shaped bacteria having ubiquinone-10 (Q-10) or ubiquinone-11 (Q-11) as the major respiratory quinone and DNA G+C contents ranging from 59.5 to 66.2mol% [1, 2, 4–13]. In addition, summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$), $C_{18:0}$ and 11-methyl-$C_{18:1}\omega7c$ and diphosphatidylglycerol (DPG) and phosphatidylglycerol (PG) have been reported as major fatty acids and polar lipids of the genus *Devosia*, respectively. In the course of our research on interactions between marine red algae and bacteria, numerous novel bacteria have been isolated from marine red algae [14–16]. In this study, we have isolated two putatively novel species within the genus *Devosia* from a marine red alga and their taxonomic properties were characterized using a polyphasic approach.

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Abbreviations: ANI, average nucleotide identity; ASW, artificial seawater; dDDH, digital DNA–DNA hybridization; DPG, diphosphatidylglycerol; MA, marine agar; MB, marine broth; ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining; PG, phosphatidylglycerol; Q, ubiquinone.
The GenBank accession numbers for the 16S rRNA gene sequences of strains G2-5^T and G20-9^T are OL985672 and OM763956, respectively, and those for the genome sequences of strains G2-5^T and G20-9^T are CP118247 and CP118246, respectively.
†These authors contributed equally to this work
Three supplementary figures and two supplementary tables are available with the online version of this article.

ISOLATION AND ECOLOGY

Strains G2-5^T and G20-9^T were isolated from the phycosphere of a *Chondrus* species, a marine red alga, collected from the Gonghyeonjin, Gangwon-do, Republic of Korea (38° 21′ 21″ N, 128° 30′ 45″ E), as described previously [15]. In brief, the collected red alga underwent a thorough wash using artificial seawater (ASW; 20.0 g NaCl, 2.9 g $MgSO_4$, 4.5 g $MgCl_2{\cdot}6H_2O$, 0.6 g KCl, 1.8 g $CaCl_2{\cdot}2H_2O$ per litre) through mechanical vortexing. Subsequently, the washed alga was homogenized mechanically using a homogenizer, followed by serial dilution in ASW. Aliquots of 100 µl from each serial dilution were plated on marine agar 2216 (MA; BD) and incubated aerobically at 25°C for 7 days. Colonies grown on MA were subjected to PCR amplification of the 16S rRNA gene using the universal primers F1 (5′-AGAGTTTGATCMTGGCTCAG-3′) and R13 (5′-TACGGYTACCTT GTTACGACTT-3′) [15]. The resulting PCR amplicons were double-digested with restriction enzymes *Hae*III and *Hha*I, and their restriction fragment patterns were analysed on 2% agarose gel. Colonies exhibiting identical restriction fragment patterns

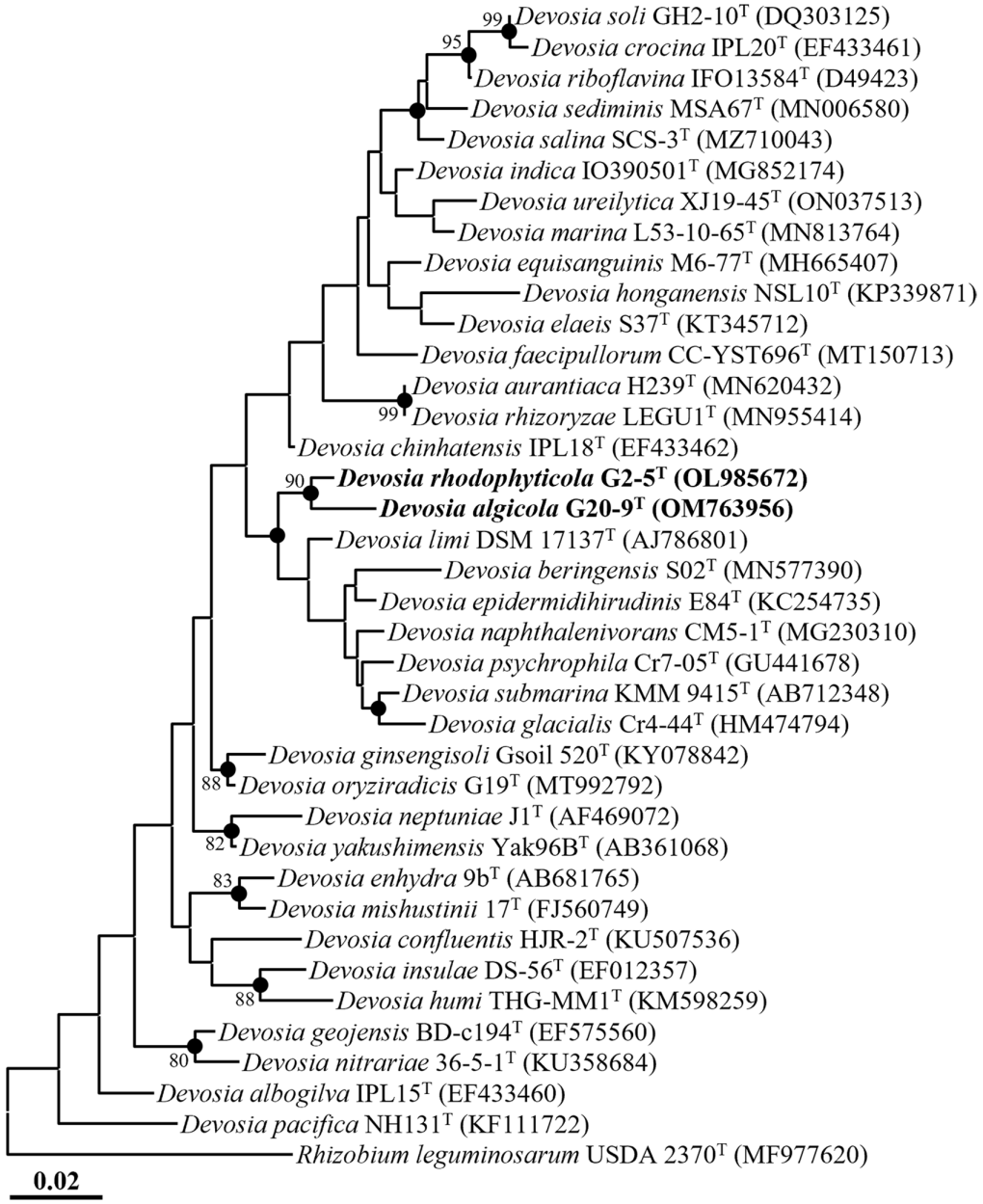

Fig. 1. Maximum-likelihood tree showing the phylogenetic relationships between strains G2-5^T and G20-9^T and their closely related taxa, based on 16S rRNA gene sequences. Only bootstrap values exceeding 70% are indicated on the nodes as percentages from 1000 replicates. Filled circles (●) denote nodes that were also retrieved in the trees constructed using the neighbour-joining and maximum-parsimony algorithms. *Rhizobium leguminosarum* USDA 2370^T (MF977620) was employed as an outgroup. Bar, 0.02 substitutions per nucleotide.

of the 16S rRNA genes were excluded from further analysis. Sequencing of representative PCR amplicons with unique fragment patterns was performed using the universal primer 340F (5′-CCTACGGGAGGCAGCAG-3′) [15] at Macrogen (Seoul, Republic of Korea). The obtained 16S rRNA gene sequences were compared with sequences of all validly published type species available in the EzBioCloud server (www.ezbiocloud.net/identify) [17].

Two putative novel strains within the genus *Devosia*, designated as strains G2-5^T and G20-9^T, were selected for further taxonomic analyses. Strains G2-5^T and G20-9^T were routinely cultured on MA for 2 days at 25°C and were stored at −80°C in marine broth (MB; BD) supplemented with 15% (v/v) glycerol for long-term preservation. *Devosia limi* KACC 11576^T, *Devosia beringensis* JCM 33772^T, *Devosia epidermidihirudinis* DSM 25750^T and *Devosia naphthalenivorans* JCM 32509^T were employed as reference strains for comparisons of fatty acid compositions, as well as genomic, and phenotypic characteristics.

16S rRNA GENE SEQUENCE PHYLOGENY

The 16S rRNA gene amplicons of strains G2-5^T and G20-9^T, generated using the F1 and R13 primers, were subsequently sequenced with the universal primers 518R (5′-ATTACCGCGGCTGCTGG-3′) and 805F (5′-GATTAGATACCCTGGTAGTC-3′ [14]. Through the assembly of sequences obtained from the 340F, 518R and 805F primer-based sequencing, nearly complete 16S rRNA gene sequences for strains G2-5^T (1423 nucleotides) and G20-9^T (1422 nucleotides) were acquired. The 16S rRNA gene sequence similarities between strains G2-5^T and G20-9^T and their closely related type strains were calculated using the nucleotide similarity search program on the EzBioCloud server [18]. The 16S rRNA gene sequences of G2-5^T and G20-9^T, along with those of their closely related type strains, were aligned, and phylogenetic trees with bootstrap values (1000 replications) were reconstructed using the maximum-likelihood (ML), neighbour-joining (NJ) and maximum-parsimony (MP) algorithms in MEGA11 software [19].

The 16S rRNA gene sequence similarity between strains G2-5^T and G20-9^T was found to be 98.2%, which falls below the cutoff value of 98.7% for designating different species, implying that these two strains may indeed represent distinct species. Comparative analysis of 16S rRNA gene sequences revealed that strain G2-5^T exhibited the highest sequence similarities of 97.7, 97.4 and 97.0% to *D. limi* DSM 17137^T, *D. epidermidihirudinis* E84^T and *D. ginsengisoli* Gsoil 520^T, respectively. Strain G20-9^T, on the other hand, showed the highest sequence similarities of 96.9, 96.8 and 96.7% to *D. beringensis* S02^T, *D. psychrophila* Cr7-05^T and *D. limi* DSM 17137^T, respectively. Phylogenetic analysis utilizing 16S rRNA gene sequences, employing the ML algorithm, demonstrated that strains G2-5^T and G20-9^T formed a distinct phylogenetic lineage within the genus *Devosia*, separate from other species members of the genus *Devosia* (Fig. 1). This phylogenetic affiliation was also corroborated by phylogenetic trees reconstructed with the MP and NJ algorithms (Fig. S1, available in the online version of this article). Thus, the analysis based on 16S rRNA gene sequences suggests that strains G2-5^T and G20-9^T belong to the genus *Devosia*.

GENOME FEATURES AND PHYLOGENY OF WHOLE GENOME SEQUENCES

Genomic DNA of strains G2-5^T and G20-9^T was extracted from cells cultured in MB using the Wizard Genomic DNA purification kit (Promega) following the manufacturer's instructions. The genomic DNA was sequenced using an Oxford Nanopore MinION sequencer (ONT), and the resulting sequencing reads were assembled *de novo* using Flye software (version 2.9.1) [20]. The assembled genomes of strains G2-5^T and G20-9^T underwent quality checks for completeness and contamination rate using CheckM2 software (version 1.0.2) [21]. The whole genome sequences of strains G2-5^T and G20-9^T were submitted to GenBank and annotated using the NCBI Prokaryotic Genome Annotation Pipeline [22]. A phylogenetic analysis utilizing the genomes of strains G2-5^T and G20-9^T, along with closely related type strains. This analysis was based on the protein sequences of their bac120 marker genes sourced from the Genome Taxonomy Database (GTDB) taxonomy [23]. The concatenation alignment of these protein sequences and the subsequent reconstruction of the phylogenomic tree were executed using MEGA11 software. Average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) values among strains G2-5^T and G20-9^T, and their closely related type strains were calculated using the Orthologous ANI Tool online (www.ezbiocloud.net/tools/orthoani) [24] and the Genome-to-Genome Distance Calculator version 3.0 (https://ggdc.dsmz.de/ggdc.php) with formula 2 [25], respectively.

The assembly of MinION sequencing reads from strains G2-5^T and G20-9^T, with average genome coverages of approximately 30× and 32×, respectively, resulted in complete genomes. The completeness and contamination rates, as determined by CheckM2, were 93.4 and 0.21% for strain G2-5^T, and 91.4 and 1.3% for strain G20-9^T, respectively. These values meet the criteria (≥90% completeness and ≤10% contamination) for high-quality genomes [21]. The assembled genomes of strains G2-5^T and G20-9^T were single contigs with sizes of 3475 and 3600 kb, respectively. The DNA G+C contents of strains G2-5^T and G20-9^T, calculated from their genomes, were 57.2 and 57.5%, respectively, slightly lower than those of other *Devosia* species [26]. The genomes of strains G2-5^T and G20-9^T contained 3398 and 3843 genes in total, respectively, which included 3212 and 3256 protein-coding genes, 57 and 56 total RNA genes, 47 and 46 tRNA genes, and six rRNA genes, respectively. The general genomic features of the strains are summarized and compared with those of closely related type strains of the genus *Devosia* in Table 1.

A phylogenomic tree, based on the bac120 marker genes, indicated that strains G2-5^T and G20-9^T form a distinct lineage within the genus *Devosia,* separate from other *Devosia* species (Fig. 2). The ANI and dDDH values between strains G2-5^T and G20-9^T

Table 1. General genomic features† of strains G2-5^T and G20-9^T and closely related *Devosia* type strains

Strains: 1, G2-5^T (CP118247); 2, G20-9^T (CP118246); 3, *D. limi* DSM 17137^T (FQVC00000000); 4, *D. beringensis* S02^T (CP045422); 5, *D. epidermidihirudinis* E84^T (LANJ00000000); 6, *D. naphthalenivorans* CM5-1^T (PYVW00000000). The genomes of strains G2-5^T and G20-9^T were sequenced in this study.

Feature	1	2	3	4	5	6
Genome status (no. of contigs)*	C (1)	C (1)	D (25)	C (1)	D (47)	D (55)
Genome size (kb)	3475	3600	4298	4049	3860	4488
G+C content (mol%)	57.2	57.5	62.7	63.8	61.1	61.4
No. of total genes	3398	3843	4125	3945	3757	4389
No. of protein-coding genes	3212	3256	4023	3857	3669	4240
No. of total RNA	57	56	51	58	59	60
No. of tRNA	47	46	44	48	49	50
No. of rRNA	6	6	3	6	6	6
No. of total CAZy† genes:						
Glycoside hydrolase	57	48	38	45	39	53
Glycosyltransferase	20	17	23	30	26	36
Polysaccharide lyase	0	0	1	1	1	0
Carbohydrate esterase	9	7	8	7	8	9
Auxiliary activities	6	2	8	4	3	7
Carbohydrate-binding module	2	1	1	2	1	3

*The bioinformatic analysis of the genomes was carried out using the NCBI prokaryotic genome annotation pipeline (www.ncbi.nlm.nih.gov/genome/annotation_prok/).
†C, complete; D, draft; CAZy, carbohydrate-active enzyme.

were 82.3 and 25.0%, respectively (Table S1). These results indicate that strains G2-5^T and G20-9^T represent different species within the genus *Devosia* [17]. The ANI and dDDH values between strains G2-5^T and G20-9^T and other *Devosia* species were lower than 73.9 and 19.2%, respectively, which fall clearly below the thresholds for prokaryotic species delineation (ANI, ~95%; dDDH, 70%) [17]. In conclusion, the phylogenomic and genomic relatedness analyses of strains G2-5^T and G20-9^T with closely related type strains provide strong evidence that they represent two distinct novel species within the genus *Devosia*.

ALGAL SYMBIOSIS AND MARINE ENVIRONMENTAL-ASSOCIATED GENES

Bacteria residing in the phycosphere of algae can impact the algal host through various bacterial–algal interactions, including the biosynthesis of substances such as vitamins and nutrients [27]. Genomic analysis of strain G2-5^T revealed that it possesses all necessary genes (*hemABCD*, *cysG, cbiABCDEFGHJKLTP* and *cobAOSU*) for the biosynthesis of cobalamin from glycine (Fig. 3a). Conversely, strain G20-9^T lacks the genes required for cobalamin biosynthesis. Moreover, both strains G2-5^T and G20-9^T are predicted to have the capacity to synthesize riboflavin from ribulose 5-phosphate (*ribBEH*) (Fig. 3b). These vitamin biosynthetic capabilities in strains G2-5^T and G20-9^T suggest potential beneficial effects on the growth of their algal hosts [28]. Microbes inhabiting marine environments employ various strategies to cope with environmental stresses, such as osmotic stress induced by high salinity [29]. The survival of bacterial cells in high salinity environments necessitates the maintenance of osmotic balance. It has been reported that marine bacteria accumulate compatible solutes internally to achieve this balance [30]. Strains G2-5^T and G20-9^T possess genes for synthesizing ectoine (*lysC*, *asd* and *ectABC*) from L-aspartate, as well as hydroxy ectoine (*ectD*) from ectoine—both of which are representative compatible solutes [29]. Consequently, strains G2-5^T and G20-9^T are expected to demonstrate tolerance and adaptation to salinity stress, particularly in marine habitats, through the biosynthesis of compatible solutes.

Bacteria residing in the phycosphere have been recognized for their capacity to degrade algal polysaccharides—a prevalent component in marine environments—and utilize algal cell wall constituents to support their growth [31]. Consequently, we conducted an examination of the Carbohydrate Active Enzyme (CAZy) genes in strains G2-5^T and G20-9^T using the dbCAN3 server based on the CAZy database [32]. Our analysis revealed that the genomes of strains G2-5^T and G20-9^T are predicted to encompass a total of 94 and 75 genes encoding various CAZys, respectively. These genes are classified into five distinct classes:

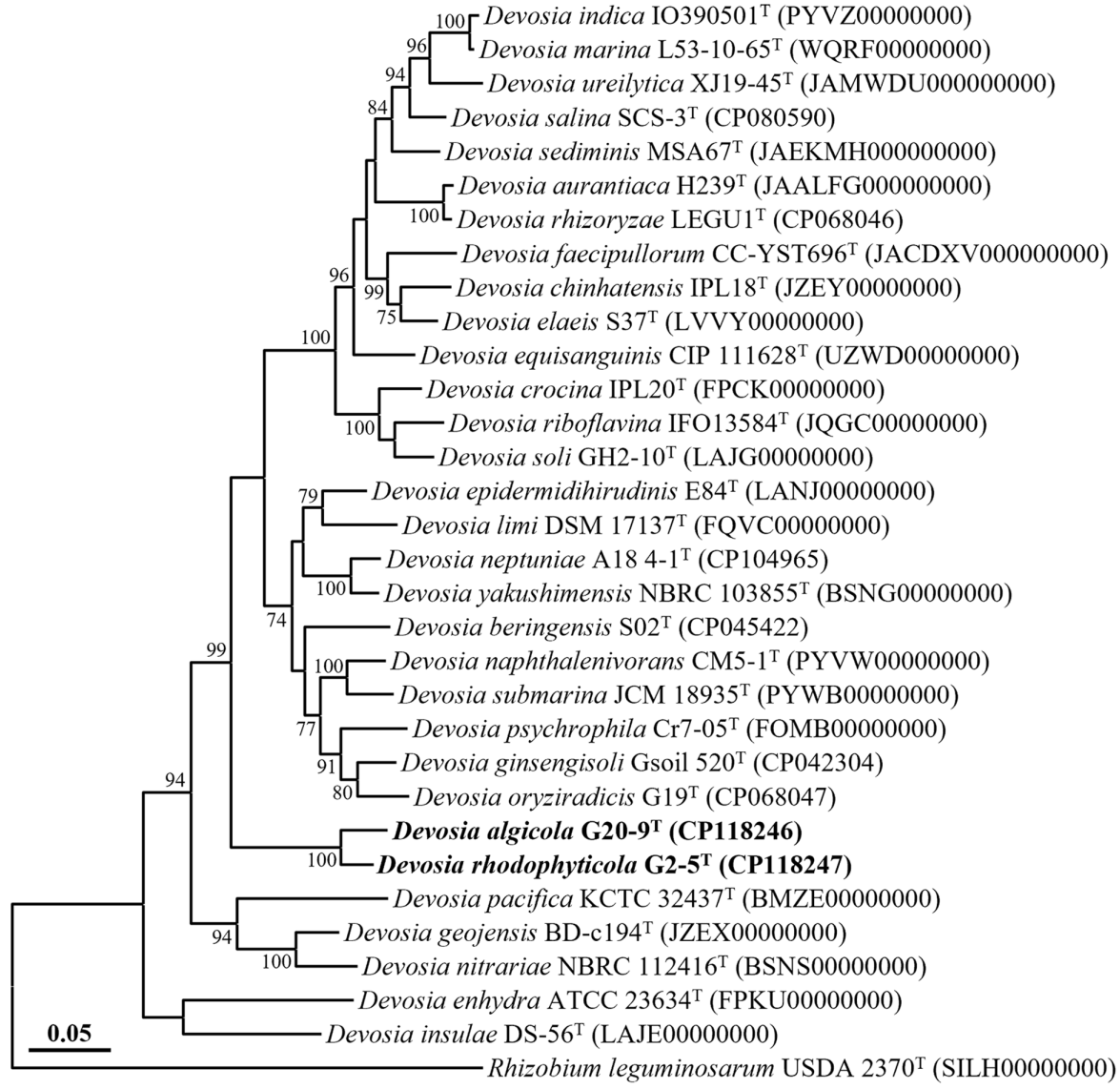

Fig. 2. Phylogenomic tree showing the phylogenetic relationships between strains G2-5^T and G20-9^T and closely related taxa, based on the bac120 marker genes of GTDB-Tk. *Rhizobium leguminosarum* USDA 2370^T (SILH00000000) was employed as an outgroup. Bar, 0.05 substitutions per nucleotide.

glycoside hydrolase (GH, 57 and 48, respectively), glycosyltransferase (GT, 20 and 17, respectively), carbohydrate esterase (CE, nine and seven, respectively), auxiliary activities (AA, six and two, respectively) and carbohydrate-binding module (CBM, two and one, respectively). Similar profiles were observed in other reference *Devosia* strains (Table 1), indicating that *Devosia* strains are likely equipped to metabolize cell wall components of red algae. The principal CAZy classes, each featuring five or more CAZy genes, included GH177, GH179, GT2 and GT51 families for strain G2-5^T, and GH177, GH179 and GT2 families for strain G20-9^T. Glycoside hydrolases GH117 and GH16 families, recognized for their activity on a wide range of polysaccharides within the marine environment [33], were identified in the genomes of strains G2-5^T (PSQ90_RS10775–85) and G20-9^T (PSQ19_RS05420), respectively. This implies that strains G2-5^T and G20-9^T, isolated from a red alga, may possess the capability to degrade a variety of macroalgal polysaccharides.

MORPHOLOGY AND PHYSIOLOGY

The growth of strains G2-5^T and G20-9^T was evaluated on various bacteriological agar media: MA, Reasoner's 2A (R2A) agar, Luria–Bertani (LB) agar, tryptic soy agar (TSA) and nutrient agar (NA), all supplemented with 2% NaCl, incubated at 30°C for 2 days. Subsequently, the growth of strains G2-5^T and G20-9^T was examined at different temperatures (10–40°C at 5°C intervals) on MA for 2 days, and at varying pH values (pH 4.0–10.0 at 1.0 pH unit intervals) in MB at 25°C for 2 days. pH

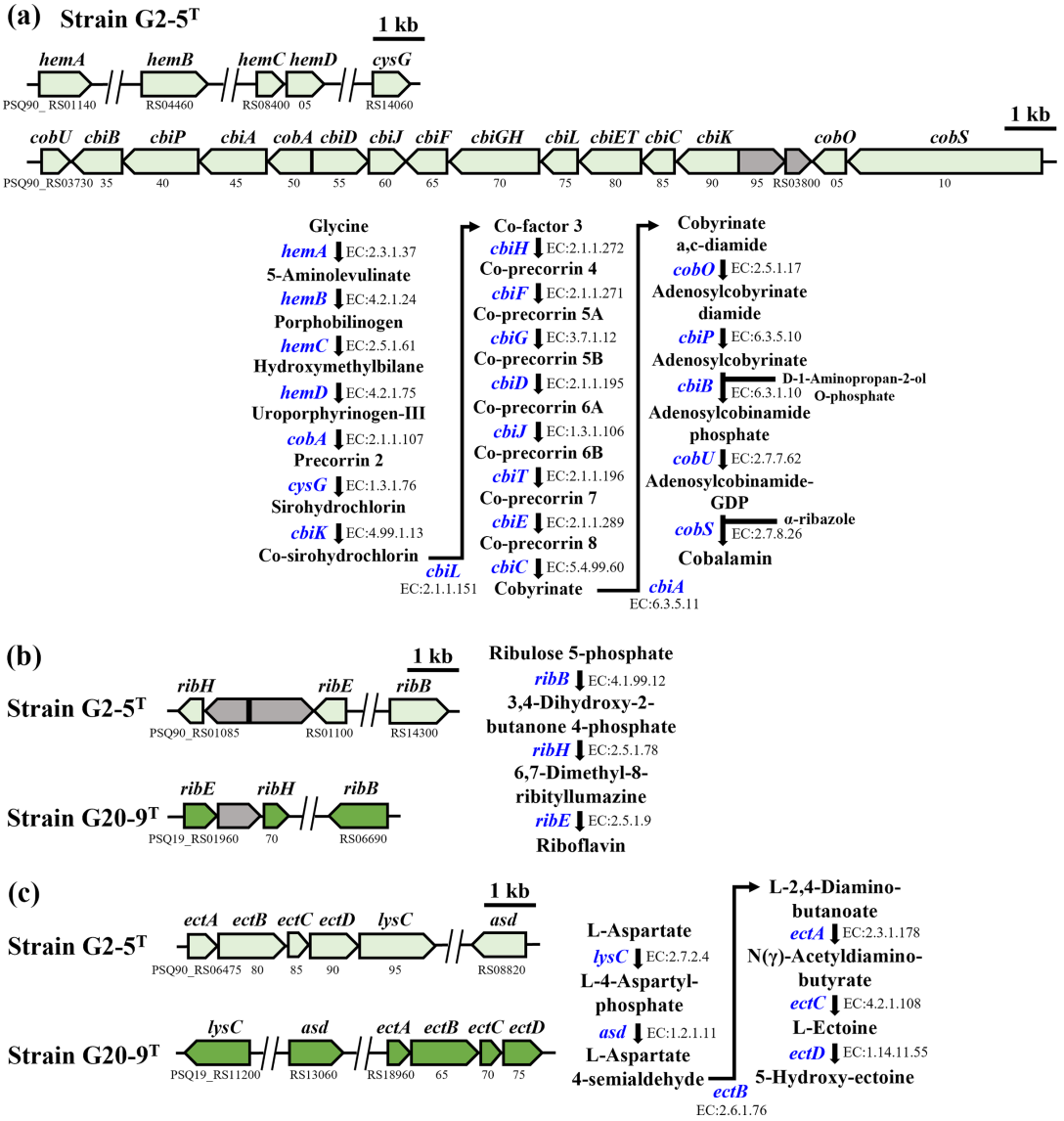

Fig. 3. Organization and putative functions of genes associated with cobalamin biosynthesis in strain G2-5^T (a) and riboflavin (b) and ectoine (c) biosynthesis in strains G2-5^T and G20-9^T. The putative functions of the genes were inferred by conducting BLAStP searches against the UniProt database. *hemA*, 5-aminolevulinate synthase; *hemB*, porphobilinogen synthase; *hemC*, hydroxymethylbilane synthase; *hemD*, uroporphyrinogen-III synthase; *cobA*, uroporphyrin-III C-methyltransferase; *cysG*, precorrin-2 dehydrogenase; *cbiK*, sirohydrochlorin cobaltochelatase; *cbiL*, cobalt-factor II C20-methyltransferase; *cbiH*, cobalt-factor III methyltransferase; *cbiF*, cobalt-precorrin-4 C11-methyltransferase; *cbiG*, cobalt-precorrin 5A hydrolase; *cbiD*, cobalt-precorrin-5B (c1)-methyltransferase; *cbiJ*, cobalt-precorrin-6A reductase; *cbiT*, cobalt-precorrin-6B (c15)-methyltransferase; *cbiE*, cobalt-precorrin-7 (c5)-methyltransferase; *cbiC*, cobalt-precorrin-8 methylmutase; *cbiA*, cobyrinate a,c-diamide synthase; *cobO*, cobalamin adenosyltransferase; *cbiP*, adenosylcobyric acid synthase; *cbiB*, adenosylcobinamide-phosphate synthase; *cobU*, adenosylcobinamide kinase; *cobS*, adenosylcobinamide-GDP ribazoletransferase; *ribE*, riboflavin synthase; *ribH*, 6,7-dimethyl-8-ribityllumazine synthase; *ribB*, 3,4-dihydroxy 2-butanone 4-phosphate synthase; *lysC*, aspartate kinase; *asd*, aspartate-semialdehyde dehydrogenase; *ectB*, diaminobutyrate-2-oxoglutarate transaminase; *ectA*, L-2,4-diaminobutyric acid acetyltransferase; *ectC*, L-ectoine synthase; *ectD*, ectoine hydroxylase.

values of MB (ranging from pH 4.0 to 10.0) were achieved using sodium citrate (pH 4.0–5.0), sodium phosphate (pH 6.0–8.0) and sodium carbonate–bicarbonate (pH 9.0–10.0) buffers [34]. Adjustments to pH, if necessary, were made with hydrogen chloride or sodium hydroxide after sterilization (121°C for 15 min). Growth at different NaCl concentrations was assessed for 2 days in MB (ranging from 0 to 6% at 0.5% intervals), which was prepared in the laboratory following the MB medium composition. To assess anaerobic growth, strains G2-5^T and G20-9^T were streaked on MA, and growth was monitored after 3 weeks of incubation at 25°C under anaerobic conditions facilitated by the GasPak Plus system (BBL).

The cellular morphology and motility of strains G2-5^T and G20-9^T were examined using a phase-contrast microscope (Zeiss Axio Scope. A1; Carl Zeiss). Furthermore, for a detailed examination of morphology and flagella, cells cultivated on MA for 2 days were affixed to formvar-coated copper grids, negatively stained with 2% (w/v) uranyl acetate (Sigma-Aldrich) for 15 s, and subsequently examined under a transmission electron microscope (JEM-1010, JEOL). Gram staining was carried out using a Gram stain kit (bioMérieux) following the manufacturer's instructions. Catalase and oxidase activities were assessed by observing oxygen bubble production in a 3% (v/v) aqueous hydrogen peroxide solution (Junsei) and the oxidation of 1% (w/v) tetramethyl-*p*-phenylenediamine (Merck), respectively [35]. The phenotypic traits of strains G2-5^T and G20-9^T were compared with reference strains under identical conditions at their respective optimal temperatures. Hydrolysis of aesculin, casein, starch, tyrosine, Tween 20 and Tween 80 was investigated on MA, as previously described [35, 36]. Additional biochemical features were assessed using the API 20NE kit from bioMérieux, following the manufacturer's instructions, with the API kit solution adjusted to approximately 2% (w/v) NaCl.

The optimal growth medium for strains G2-5^T and G20-9^T was MA, with relatively favourable growth also observed on NA and R2A media containing 2% NaCl. While strain G2-5^T exhibited robust growth on LB agar containing 2% NaCl, strain G20-9^T displayed slower growth. Both strains demonstrated limited growth on TSA containing 2% NaCl. Cells of strains G2-5^T and G20-9^T were Gram-stain-negative, motile rods, each possessing a single flagellum (0.6–0.8 µm wide and 1.5–1.6 µm long; 0.6–0.9 µm wide and 1.6–1.9 µm long, respectively; Fig. S2). Anaerobic growth was not observed for strains G2-5^T and G20-9^T, indicating their strict aerobic nature. Many phenotypic characteristics of strains G2-5^T and G20-9^T, including motility, oxidase and catalase activity, *β*-galactosidase and arginine dihydrolase activity, indole production, hydrolysis of aesculin, casein, gelatin, starch, Tween 20 and Tween 80, as well as assimilation of capric acid, were consistent with those of closely related reference type strains (Table 2).

CHEMOTAXONOMIC CHARACTERISTICS

The respiratory isoprenoid quinones of strains G2-5^T and G20-9^T were extracted and analysed using an HPLC system (model LC-20A, Shimadzu) equipped with a reversed-phase column (250×4.6 mm, Kromasil, Akzo Nobel) and a diode array detector (SPD-M20A, Shimadzu) with a methanol–isopropanol eluent (2:1, v/v) at a flow rate of 1 ml min^{-1}, as previously detailed [37]. For cellular fatty acid analysis, strains G2-5^T and G20-9^T, along with reference strains, were cultivated aerobically in MB at 25°C and harvested at the exponential growth phase (optical density, OD_{600}, 0.7–0.8). Fatty acids were extracted from the microbial cells using the standard MIDI protocol, involving saponification, methylation and extraction. Subsequently, the cellular fatty acids were analysed using a gas chromatograph (Hewlett Packard 6890) and identified using the RTSBA6 database of the Microbial Identification System (Sherlock version 6.0B) [38]. Polar lipids of strains G2-5^T and G20-9^T were analysed using two-dimensional thin-layer chromatography, with cells harvested during the exponential growth phase, following the procedure described by Minnikin *et al.* [39]. Various reagents were employed to detect different polar lipids, including 10% ethanolic molybdophosphoric acid (for total polar lipids), ninhydrin (for aminolipids), Dittmer–Lester (for phospholipids) and *α*-naphthol/sulphuric acid (for glycolipids). PG and DPG identified from the polar lipid analysis were validated using standard chemicals purchased from Sigma-Aldrich.

The respiratory isoprenoid quinones detected in strains G2-5^T and G20-9^T were identified as Q-10, which aligns with the predominant respiratory quinone observed in other species of the genus *Devosia* [4–12]. Regarding major fatty acids (those constituting >10% of the total fatty acids), both strains exhibited summed feature 8 (comprising $C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c*), 11-methyl-$C_{18:1}$ *ω7c* and $C_{18:0}$. The overall fatty acid profiles of strains G2-5^T and G20-9^T, as well as those of reference *Devosia* species, were generally similar, with only slight variations in the respective proportions of fatty acids (Table S2). In strain G2-5^T, the predominant polar lipids were identified as PG, DPG, along with two unidentified glycolipids. In strain G20-9^T, the major polar lipids comprised PG, DPG and four unidentified lipids (Fig. S3). These polar lipid profiles of strains G2-5^T and G20-9^T were in line with those observed in other *Devosia* species [2, 4–11].

TAXONOMIC CONCLUSION

In conclusion, the results of our phylogenetic, genomic relatedness, phenotypic, physiological, biochemical and chemotaxonomic analyses support the identification of strains G2-5^T and G20-9^T as representing two novel species of the genus *Devosia*, for which we propose the following names: *Devosia rhodophyticola* sp. nov. and *Devosia algicola* sp. nov., respectively.

DESCRIPTION OF *DEVOSIA RHODOPHYTICOLA* SP. NOV.

Devosia rhodophyticola (rho.do.phy.ti′co.la. N.L. neut. pl. n. *Rhodophyta*, the division of the red algae; L. suffix. *-cola* (from L. masc. or fem. n. *incola*) inhabitant, dweller; N.L. fem. n. *rhodophyticola*, inhabitant of *Rhodophyta*).

Colonies grown on MA exhibit a creamy appearance, with a circular and smooth morphology. Cells are Gram-stain-negative, obligately aerobic, and motile rods, demonstrating oxidase- and catalase-positive activities. Growth occurs at 15–35°C

Table 2. Differential phenotypic characteristics between G2-5^T and G20-9^T and closely related type strains of the genus *Devosia*

Strains: 1, G2-5^T (this study); 2, G20-9^T (this study); 3, *D. limi* KACC 11576^T [10]; 4, *D. beringensis* JCM 33772^T [4]; 5, *D. epidermidihirudinis* DSM 25750^T [13]; 6, *D. naphthalenivorans* JCM 32509^T [5]. All strains are positive for the following characteristics: flagellum motility, activity* of catalase, oxidase and *β*-galactosidase, and hydrolysis* of aesculin. All strains are negative for the following characteristics: indole production*, hydrolysis* of starch, casein, gelatin, Tween 20 and Tween 80, activity* of arginine dihydrolase, and assimilation* of capric acid. +, Positive; –, negative; w, weakly positive; na, not available.

Characteristic	1	2	3	4	5	6
Isolation source	Marine alga	Marine alga	Nitrifying inoculum	Sediment	Medical leech	Sediment
Colony colour	Cream	Cream	White	White	Beige	Cream
Range for growth:						
Temperature (°C)	15–35	15–30	na	4–25	4–30	5–30
pH	5.0–9.0	6.0–8.0	na	5.5–9.0	5–11.5	6.0–8.0
NaCl (%)	0–4.5	0–3.5	na	0–7	0–5	0–5
Nitrate reduction	–	–	–	–	+	–
Glucose fermentation	–	–	–	–	+	+
Hydrolysis* of:						
Urease	+	–	–	–	–	–
Tyrosine	–	–	–	–	+	–
Assimilation* of:						
D-Glucose	+	–	w	+	+	+
L-Arabinose	+	+	w	–	–	+
D-Mannose	w	+	w	–	–	+
D-Mannitol	+	–	w	+	+	+
N-Acetyl-glucosamine	+	–	w	+	w	w
Maltose	+	–	w	+	+	+
Potassium gluconate	+	–	–	+	w	w
Adipic acid	–	–	–	+	–	–
Malic acid	+	–	–	+	–	–
Trisodium citrate	w	–	–	+	w	–
Phenylacetic acid	w	–	–	+	–	–

*Data were obtained from this study.

(optimum, 30°C) and pH 5.0–9.0 (optimum, pH 5.0–6.0) and in the presence of 0–4.5% (w/v) NaCl (optimum, 0.5–1.0%). Hydrolysis of aesculin and urea is positive, but hydrolysis of tyrosine, casein, starch, gelatin, Tween 20 and Tween 80 is negative. Nitrate is not reduced to nitrite. Indole production and D-glucose fermentation are negative. Positive for *β*-galactosidase activity, but negative for arginine dihydrolase activity. Positive for assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, maltose, potassium gluconate, malic acid, trisodium citrate and phenylacetic acid, but negative for assimilation of capric acid and adipic acid. The major respiratory isoprenoid quinone detected is Q-10 and the major cellular fatty acids comprise summed feature 8 ($C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c*), 11-methyl-$C_{18:1}$ *ω7c* and $C_{18:0}$. PG, DPG and two unidentified glycolipids are detected as major polar lipids.

The type strain is G2-5^T (=KACC 22601^T=JCM 35404^T), isolated from the phycosphere of a *Chondrus* species, a marine red alga, collected in the Republic of Korea. The genome size and DNA G+C content of the type strain are 3475 kb and 57.2 mol% (calculated from the whole genome sequence), respectively. The GenBank accession numbers of the 16S rRNA gene and genome sequences of strain G2-5^T are OL985672 and CP118247, respectively.

DESCRIPTION OF *DEVOSIA ALGICOLA* SP. NOV.

Devosia algicola (al.gi′co.la. L. fem. n. *alga*, an alga, a seaweed; L. suffix. *-cola* (from L. masc. or fem. n. *incola*) inhabitant, dweller; N.L. fem. n. *algicola*, dweller of an alga).

Colonies grown on MA exhibit a creamy appearance, with a circular and smooth morphology. Cells are Gram-stain-negative, obligately aerobic, and motile rods, demonstrating oxidase- and catalase-positive activities. Growth occurs at 15–30°C (optimum, 25°C) and pH 6.0–8.0 (optimum, pH 6.0) and in the presence of 0–3.5% NaCl (optimum, 0.5–1.5%). Hydrolysis of aesculin is positive, but hydrolysis of tyrosine, urea, casein, starch, gelatin, Tween 20 and Tween 80 is negative. Nitrate is not reduced to nitrite. Indole production and D-glucose fermentation are negative. *β*-Galactosidase activity is positive, but arginine dihydrolase activity is negative. Assimilation of L-arabinose and D-mannose is positive, but assimilation of D-glucose, D-mannitol, *N*-acetyl-glucosamine, maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid is negative. The major respiratory isoprenoid quinone detected is Q-10 and the major cellular fatty acids comprise summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$), 11-methyl-$C_{18:1}\omega7c$ and $C_{18:0}$. PG, DPG and four unidentified lipids are detected as major polar lipids.

The type strain is G20-9^T (=KACC 22650^T=JCM 35405^T), isolated from the phycosphere of a *Chondrus* species, a marine red alga, collected in the Republic of Korea. The genome size and DNA G+C content of the type strain are 3600 kb and 57.5mol% (calculated from the whole genome sequence), respectively. The GenBank accession numbers of the 16S rRNA gene and genome sequences of strain G20-9^T are OM763956 and CP118246, respectively.

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Conflicts of interest
The authors declare that there are conflicts of interest.

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