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Devosia rhizoryzae sp. nov., and *Devosia oryziradicis* sp. nov., novel plant growth promoting members of the genus *Devosia*, isolated from the rhizosphere of rice plants[§]

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Two novel Gram-negative, aerobic, asporogenous, motile, rod-shaped, orange and white pigmented, designated as LEGU1^T and G19^T, were isolated from the roots of rice plants, collected from Goyang, South Korea. Phylogenetic analysis based on their 16S rRNA gene sequences revealed that they belonged to the genus *Devosia* and formed a different lineage and clusters with different members of the genus *Devosia*. These strains shared common chemotaxonomic features. In particular, they had Q-10 as the sole quinone, phosphatidylglycerol, diphosphatidylglycerol as the principal polar lipids and $C_{16:0}$, $C_{18:1}$ *ω7c* 11-methyl and summed feature 8 (comprising $C_{18:1}$ *ω7c*/$C_{18:1}$ *ω6c*) as the main fatty acids. The draft genome sequences of strains LEGU1^T and G19^T were 3,524,978 and 3,495,520 bp in size, respectively. Their average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) values were 72.8–81.9% and 18.7–25.1%, respectively, with each other and type strains of related species belonging to the genus *Devosia*, suggesting that these two strains represent novel species. The G + C content of strains LEGU1^T and G19^T were 62.1 and 63.8%, respectively. Of the two strains, only LEGU1^T produced carotenoid and flexirubin-type pigment. Both strains produced siderophore and indole acetic acid (IAA) in the presence of L-tryptophan. Siderophore biosynthesis genes, auxin responsive genes and tryptophan biosynthesis genes were present in their genomes. The present study aimed to determine the detailed taxonomic positions of the strains using the modern polyphasic approach. Based on the results of polyphasic analysis, these strains are suggested to be two novel bacterial species within the genus *Devosia*. The proposed names are *D. rhizoryzae* sp. nov., and *Devosia oryziradicis* sp. nov., respectively. The plant growth promoting effects of these strains suggest that they can be exploited to improve rice crop productivity. The type strain of *D. rhizoryzae* is LEGU1^T (KCTC 82712^T = NBRC 114485^T) and *D. oryziradicis* is G19^T (KCTC 82688^T = NBRC 114842^T).

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Introduction

The genus *Devosia* has been created by the reclassification of *Pseudomonas riboflavina* as *Devosia riboflavina* (Nakagawa *et al.*, 1996). As of the time of writing, the genus *Devosia* comprises 27 species with validly published names, which can be found in the up-to-date LPSN (https://lpsn.dsmz.de/genus/devosia). The genus accommodates Gram-negative, rod-shaped, flagellated, obligately aerobic, and contains Q-10 or Q-11 as the predominant respiratory quinone. The DNA G + C contents of bacteria of the genus *Devosia* range from 59.5 to 66.2 mol% (Yoo *et al.*, 2006). Members of genus *Devosia* have diverse colony colours, yellow-brown, yellow, white, cream and orange. *Devosia neptuniae* has been found to nodulate and fix nitrogen in symbiosis with *Neptunia natans*, an aquatic legume (Rivas *et al.*, 2003). Various isolation sources have been reported for the genus *Devosia* including hexachlorocyclohexan dumpsite (Dua *et al.*, 2013), deep-sea sediment (Jia, 2014), nitrifying inoculum (Vanparys *et al.*, 2005), ginseng cultivation soil (Quan *et al.*, 2020), beach sediments (Lee, 2007), glacier (Zhang *et al.*, 2012), and even on the surface of a medical leech (Galatis *et al.*, 2013). Members of the genus are best studied for their potential to degrade several toxic compounds, establishing their promising candidature for bioremediation (Wang *et al.*, 2019; Talwar *et al.*, 2020). To the best of our knowledge, no member of the genus *Devosia* has been reported to produce siderophore and auxin so far. In the present study, we isolated two novel siderophore and IAA producing strains from the roots of rice plants and examined their taxonomic positions by a polyphasic approach including genome analysis. Microbial siderophores have been known to facilitate heavy metal sequestration and play a vital role in organic compound degradation (Burd *et al.*, 2000). The use of siderophores and IAA producing bacterial strains as biofertilizers may solve the problems caused by chemical fertilizers such as heavy cost and environmental pollution (Cleland *et al.*, 1995).

Materials and Methods

Ecology of type strains

Rice requires more water than other crops and most rice varieties show better growth and produce higher yields when

grown under flooded conditions, than when grown in dry soils (Toral *et al.*, 2012). This reduces the need for herbicides and pesticides. In addition, residual herbicides and pesticides from previous seasons dissolve in water. Most weeds do not grow in flooded fields, this reduces the growth of robust pests (Ueji and Inao, 2001; Bandumula, 2018). The rice-growing season starts in late April in South Korea. On May 2019, the roots of rice plants were collected for our study and the type strains LEGU1^T and G19^T were isolated from the submerged rhizosphere of a rice plant. The field was flooded a few weeks before sampling of the rhizosphere. For water supply, drainage was closely linked to irrigation and water passed from one plot to another with the help of a motor pump (Supplementary data Fig. S1). This suggests that the type strains were living in association with rice roots.

Isolation, culture conditions and maintenance of strains

In continuation to our previous study that aimed to assess the bacterial communities and explore the novel strains present in the roots of rice plants, samples were collected from a paddy field near Dongguk University, Goyang, South Korea (GPS positioning of the sample collection site; 37° 40′ 26.4″ N 126° 48′ 20.88″ E). The root samples were collected in sterile polyethylene bags and prepared for screening of novel isolates as described previously (Chhetri *et al.*, 2020). The macerated samples were serially diluted using 0.85% NaCl. Isolation was performed using R2A agar (Difco) at 28°C for 1 week. Different colonies were chosen according to their morphology and color. The colonies were then purified by transferring to new R2A agar plates. The purified colonies were sent to Bionics for 16S rRNA gene analysis. From the purified bacterial colonies, two novel strains were identified to be members of the genus *Devosia*. For routine assessment, short-term maintenance and long-term preservation were performed as described previously (Kim *et al.*, 2019a). The two novel strains were designated as LEGU1^T and G19^T. The strains were preserved in R2A broth (Difco) supplemented with 50% (v/v) glycerol at -80°C. Both strains were deposited at the Korean Agricultural Culture Collection (KACC 21745^T) and Biological Resource Centre, NITE (NBRC 114484^T).

Phylogenetic analysis

Genomic DNA was extracted using the TaKaRa MiniBEST Bacteria Genomic DNA extraction Kit version 3.0 (TaKaRa) according to the manufacturer's instructions. The 16S rRNA genes of the strains were directly amplified by colony-PCR using the universal bacterial primer pairs 27F, 518F, 805R, and 1492R. The PCR products were commercially sequenced (Solgent). The 16S rRNA gene sequences of the closest species were then aligned with those of the novel strains using ClustalX version 2.0 (Thompson *et al.*, 1997). The alignment was manually verified and adjusted before reconstructing the phylogenetic trees. The phylogenetic placement of the strains was then inferred using neighbour-joining (NJ) (Saitou and Nei, 1987), maximum-likelihood (ML) (Felsentein, 1981) and maximum-parsimony (MP) (Fitch, 1971) methods as implemented in the program mega 7.0 (Kumar *et al.*, 2016). For the NJ method, distances were calculated using the Kimura two-parameter model (Kimura, 1980), while implementing the complete deletion option. Tree topology stability was evaluated by bootstrap analysis based on 1,000 resamplings (Felsentein, 1985).

Morphology, phenotypic characteristics and biochemical features

The cell morphology of strains LEGU1^T and G19^T was visualized under a transmission electron microscope (TEM) (LIBRA 120, Carl Zeiss), using cells grown in R2A broth at 30°C for four days. To prepare cells for microscopic analysis, they were suspended in distilled water (DW). A grid was placed on the suspension for one min followed by negative staining with phosphotungustic acid (PTA). Washing twice with distilled water for 2 sec and drying for 3 min. The optimum growth conditions of the strains LEGU1^T and G19^T were determined by culturing under different conditions (temperatures, media, pH levels and salinity levels). The temperature, pH and NaCl tolerance required for the growth of each strain were determined using optimum media. Growth at different temperatures (4, 10, 15, 20, 25, 30, 35, 40, and 45°C) was assessed after 5 days of incubation. Growth at various concentrations of NaCl (0–0.5 and 1.0–10.0%, at increments of 1.0%, w/v) was tested in R2A medium at pH 7.0 for 10 days at 30°C. Growth experiments were performed on several media such as, R2A, marine, nutrient, tryptic soy and Luria-bertani agar at 30°C for seven days. The pH range for growth was determined by cultivation at 30°C in R2A broth adjusted to pH 4–10 (at pH 1 unit intervals) before sterilization with citrate/NaH_2PO_4 buffer (pH 4.0–5.0), phosphate buffer (pH 6.0–8.0) and Tris buffer (pH 9.0–10.0) as described previously (Kim *et al.*, 2019b). Anaerobic growth was assessed by checking for colony formation on R2A agar at 25°C for 10 days in a GasPak jar (BBL). Oxygen absorber stripes (Mitsubishi Gas Chemical Company) was used to remove oxygen. The phenotypic characteristics of the strains such as Gram staining, motility, oxidase and catalase activities were determined using standard microbiological methods as described previously (Chhetri *et al.*, 2019a). The presence of flexirubin-type pigments was examined using the bathochromatic shift test with 20% (w/v) KOH solution (Fautz and Reichenbach., 1980). Cells were extracted for carotenoid analysis using a 10 ml methanol/acetone mixture (1:1; v/v) and the absorption spectrum (350–750 nm) of the pigments was assessed with a spectrophotometer (Multiskan GO; Thermo Fisher Scientific) (Chhetri *et al.*, 2019b). The hydrolysis of carboxymethyl-cellulose (CMC) was tested by adding a solution of 0.2% Congo red on R2A supplemented with 0.1% CMC. The hydrolysis of chitin, starch and casein was determined as described previously (Kim *et al.*, 2019c). Additional enzyme activities, biochemical features and physiological characteristics were tested using the API ZYM and API 20NE kits (bioMérieux) according to the manufacturer's instructions.

Genome sequencing and assembly

To further determine the taxonomic positions of the two novel strains, genome sequencing was performed using the Illumina HiSeq 4000 platform with 150 bp paired-end reads according to the manufacturer's protocols. Short reads were assembled using SPAdes analysis v.3.10.1 at Macrogen. The

locations of protein encoding genes were predicted and their functions were annotated. Prokka (v1.14.6) was used to predict the location while BLAST and the evolutionary genealogy of genes: Nonsupervised Orthologus Groups (eggNOG) 4.5 database (Huerta-Cepas *et al*., 2017) were used to determine the function and to identify the assembled sequences against the nucleotide and protein sequences database. EggNOG proportions of novel strains were compared with each other and with their reference strains. The genomic DNA G + C content was determined directly from the draft genome sequence. The CheckM bioinformatics tool was used to assess genome contamination and completeness (https://ecogenomics.github.io/CheckM) of both strains (Parks *et al*., 2015). A phylogenomic tree was reconstructed on the basis of concatenation of 92 core genes from the closest relatives of strain LEGU1^T and G19^T in the genus *Devosia* (Na *et al*., 2018). The average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) values among the strains LEGU1^T and G19^T and reference strains were calculated using an online ANI calculator and the Genome-to Genome Distance Calculator 2.1 (Meier-Kolthoff *et al*., 2013; Yoon *et al*., 2017). Gene annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (Tatusov *et al*., 2000). The result of the two novel strains was compared.

Chemotaxonomic characterization

Respiratory quinone was extracted using chloroform/methanol (2:1; v/v), evaporated under vacuum, re-extracted using acetone and analyzed using high-performance lipid chromatography (HPLC) as described previously (Collins and Jones, 1981). To analyze polar lipids and quinones, the cells were incubated in R2A medium for 5 days at 30°C and extracted as described previously (Minninkin *et al*., 1984; Komagata *et al*., 1988). Polar lipids were analyzed by two-dimensional thin-layer chromatography using chloroform/methanol/water (65:25:4; v/v/v) in the first dimension and chloroform/methanol/acetic acid/water (80:15:12:4; v/v/v/v) in the second dimension. Appropriate detection reagents (Komagata and Suzuki, 1988) were used to identify the spots; molybdophosphoric acid (phosphomolybdic acid reagent, 5% v/v solution in ethanol; Sigma-Aldrich) was used to detect total lipids, ninhydrin reagent (0.2% solution; Sigma life Science) to detect amino lipids, Zinzadze reagent (molybdenum blue spray reagent, 1.3%; Sigma Life Science) to detect phospholipids, and α-naphthol reagent was used to detect glycolipids.

Accession numbers

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA sequences of strains LEGU1^T and G19^T are MN955414

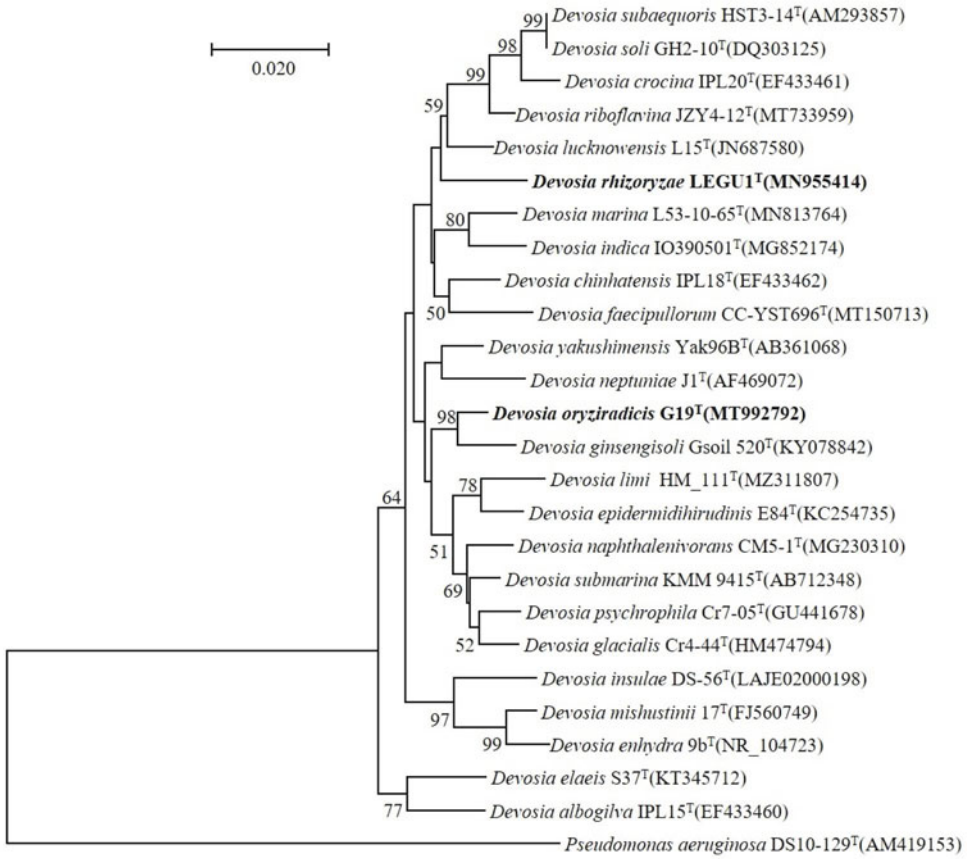

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the relationship between strains strains LEGU1^T and G19^T and other closely related members. Numbers at nodes are levels of bootstrap support (> 50%) based on 1,000 resamplings. *Pseudomonas aeruginosa* DS10-129^T (AM419153) was used as an out-group. GenBank accession numbers are given in parenthesis. Bar, 0.020 substitutions per nucleotide position.

and MT992792, respectively. The GenBank/EMBL/DDBJ accession numbers for the complete genome sequences of strains LEGU1^T and G19^T are CP068046 and CP068047, respectively.

IAA and siderophore production

Strains LEGU1^T and G19^T were grown in R2A broth with or without 0.1% tryptophan at 30°C for 5 days. The cells were centrifuged at 6,000 rpm for 30 min after 5 days of incubation. The supernatant was reserved and 1 ml was mixed with 2 ml of Salkowski's reagent (2% 0.5 $FeCl_3$ in 35% $HClO_4$ solution), incubated at room temperature for 30 min, and spectrophotometrically assessed at 530 nm. IAA production was quantified using a standard curve with known concentrations of pure commercial IAA. Uninoculated R2A broth was treated as a negative control. IAA production was indicated by change in color pink. The results were compared with and without L-tryptophan. The strains were examined for siderophore production on Chrome Azurol S (CAS) plates. The strains LEGU1^T and G19^T were spot inoculated and incubated at 30°C for 5 days.

Results and Discussion

Phylogenetic analysis

The nearly full-length of 16S rRNA gene sequences of the strains LEGU1^T and G19^T were 1,423 bp and 1,417 bp, respectively. Assessement of 16S rRNA gene sequence similarities and phylogenetic analysis revealed that both strains clustered with members of the genus *Devosia*. Strain LEGU1^T showed the highest 16S rRNA gene sequence similarities to *D. lucknowensis* L15^T (97.9%) and *D. chinhatensis* IPL18^T (97.6%), but formed an independent lineage next to *D. lucknowensis* L15^T and *D. riboflavina* JZY4-12^T within the genus *Devosia*. Consistent with the high level of 16S rRNA gene sequence similarity, strain G19^T formed an independent lineage with its most close relative *D. ginsengisoli* Gsoil 520^T (98.6%). The 16S rRNA gene phylogenetic trees obtained by the NJ, ML and MP algorithms consistently showed identical topologies regarding the two novel strains of *Devosia* (Fig. 1; Supplementary data Figs. S2 and S3). Reference strains were selected on the basis of 16S rRNA gene sequence similarities and phylogenetic analysis. *D. lucknowensis* DSM 25398^T, *D. soli* KACC 11509^T, *D. crocina* KACC 14589^T, *D. subaequoris* KACC 14985^T, *D. riboflavina* KACC 11387^T, and *D. ginsengisoli* KACC 19440^T were purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSM) and Korean Agricultural Culture Collection (KACC) and were considered for comparative analysis.

Morphology, phenotypic characteristics and biochemical features

Cells of strain LEGU1^T and G19^T were aerobic, Gram-stain negative, motile by flagella, rod-shaped and asporogenous (Supplementary data Fig. S4). Strain LEGU1^T was orange pigmented, catalase positive and oxidase negative. Strain G19^T was white pigmented and found to be positive for catalase and oxidase activities. Both strains were negative for hydrolysis of CM cellulose, starch, casein, and chitin. Anaerobic growth was not observed in both strains. Strain LEGU1^T was able to produce carotenoid (absorption maxima at 471 nm) and flexirubin-type pigments. All the strains were positive for oxidase activity, except for strain LEGU1^T, *D. crocina* KACC 14589^T and *D. soli* KACC 11509^T. The presence and absence of pigments in cells differentiated the novel strains from each other

Table 1. Average Nucleotide Identity (ANIu) and digital DNA-DNA Hybridization among strains LEGU1^T and G19^T and closely related *Devosia* members
Taxa: 1, LEGU1^T; 2, G19^T.

Strains	Accession number	OrthoANIu%		dDDH%	
		1	2	1	2
LEGU1^T	CP068046		76.2		20.2
G19^T	CP068047	76.2		20.2	
Devosia lucknowensis L15^T	FXWK00000000	76.3	75.4	20.6	20
Devosia riboflavina IFO13584^T	JQGC00000000	77.1	76.2	21.5	20.4
Devosia soli GH2-10^T	LAJG00000000	76.8	75.6	20.9	20
Devosia subaequoris DSM 23447^T	JACIEW000000000	76.9	75.8	20.4	19.5
Devosia crocina IPL20^T	FPCK00000000	77.8	76.3	21.3	20.3
Devosia submarina JCM 18935^T	PYWB00000000	75.3	75.8	20.1	20.9
Devosia epidermidihirudinis E84^T	LANJ00000000	75.1	78.1	19.9	21.5
Devosia elaeis S37^T	LVVY00000000	78.7	77.4	21.8	20.7
Devosia psychrophila CGMCC 1.10210^T	FOMB00000000	75.7	79.8	20.1	22.9
Devosia limi DSM 17137^T	FQVC00000000	75.9	78	20.2	21.4
Devosia indica IO390501^T	PYVZ00000000	77.4	75.9	20.8	19.8
Devosia naphthalenivorans CM5-1^T	PYVW00000000	75.5	78.6	20.1	22.1
Devosia chinhatensis IPL18^T	JZEY00000000	76.6	76.2	21	20.9
Devosia insulae DS-56^T	LAJE00000000	73.3	73.8	18.9	19.3
Devosia enhydra ATCC 23634^T	FPKU00000000	72.8	73.4	18.7	19.4
Devosia geojensis BD-c194^T	JZEX00000000	74.6	75.8	19.7	20.1
Devosia marina L53-10-65^T	WQRF00000000	77.1	75.9	20.7	19.8
Devosia ginsengsoli Gsoil 520^T	CP042304	76.6	81.9	20.7	25.1

and their closely related members. Only *D. crocina* KACC 14589^T and *D. soli* KACC 11509^T were positive for hydrolysis of urease. The other differentiating phenotypic and biochemical features of all the analyzed strains are provided in the species description and illustrated along with reference strains in Table 2.

Genome annotation and analysis

The draft genome of strains LEGU1^T and G19^T consisted of 1 contigs, yielding a total length 3,524,978 and 3,495,520 bp, respectively. The G + C genomic content of strains LEGU1^T and G19^T was 63.7 and 63.8 mol%, respectively which is in agreement with the findings in other species within the genus *Devosia*. A total of 3,419 and 3,420 coding sequences (CDSs) were predicted for strains LEGU1^T and G19^T, respectively. Moreover, a total of 45 tRNA genes were predicted for strains LEGU1^T and G19^T. Three rRNA genes, were found in both strains. The genomes of strain LEGU1^T and G19^T had circular chromosomes. The genome completeness values of strains LEGU1^T and G19^T were 98.9% and 99.2%, respectively; and the contamination levels were 1.1% and 0%, respectively. Thus, the draft genomes had sufficiently high quality for further analysis (Chun *et al.*, 2018). The phylogenomic tree constructed on the basis of concatenation of 92 core genes revealed that strain LEGU1^T formed a separate lineage within members of the genus *Devosia* and strain G19^T formed a distinct monophyletic clade with its closest relative (Fig. 2), these findings are consistent with those from other phylogenetic trees. EggNOG analysis of two novel strains, denoted that a total of 1,095 genes for LEGU1^T and 1,985 genes for G19^T were assigned to 24 functional categories. Among the obtained functional groups, the cluster for [E] (amino acid transport and metabolism; 266 genes for LEGU1^T and 262 genes for G19^T), constituted the largest functional group in both strains. The

Table 2. Physiological and biochemical characteristics of strains LEGU1^T and G19^T and their closely related type strains of the genus *Devosia*
Taxa: 1, strain LEGU1^T; 2, strain G19^T; 3, *D. lucknowensis* DSM 25398^T; 4, *D. riboflavina* KACC 11387^T; 5, *D. soli* KACC 11509^T; 6, *D. subaequoris* KACC 14985^T; 7, *D. crocina* KACC 14589^T; 8, *D. ginsengisoli* KACC 19440^T. Data were taken from this study unless otherwise indicated. +, positive; –, negative. -, not detected.

Characteristics	1	2	3	4	5	6	7	8
Isolation source	Plant	Plant	HCN contaminated pond soil	Soil	Green house soil	Deep sea surface	HCN dump site	Ginseng field
Colony colour	Orange	White	Orange	Cream	Light beige	Orange-red	Orange	Ivory
Cell shape	Rod	Rod	Rod	Rod	Rod	Rod	Oval	Rod
Catalase/oxidase	+/-	+/+	+/+	+/+	+/-	+/+	+/-	
Temperature range for growth (°C)	4–40	7–38	5–37	5–38	10–37	5–38	5–32	18–37
NaCl range for growth	0–4	0–5	0–3	0–3	0–5	0–5	0–3	0–0.5
pH range for growth	6.0–9.0	5.0–9.0	6.0–9.0	5.0–9.0	4.0–8.0	6.0–9.0	5.0–9.0	6.0–8.0
Media for optimum growth	R2A	R2A	R2A	R2A, TSA	R2A	TSA, MA	LB, TSA	R3A
Glucose fermentation	-	+	+	+	-	+	-	-
Indole production	+	+	-	+	-	-	-	-
Arginine dihydrolase	+	+	-	+	-	+	-	-
Hydrolysis of:								
Urease	+	+	-	+	+	-	+	-
Esculin	+	-	+	-	-	-	+	+
Gelatin	+	-	-	-	-	-	-	-
Assimilation (API 20NE) of:								
D-Glucose	+	+	+	-	+	+	-	+
L-Arabinose	-	+	+	+	+	+	-	-
D-Mannose	-	+	+	+	-	+	-	-
D-Mannitol	-	+	+	-	-	-	+	+
N-Acetyl-D-glucosamine	+	+	-	+	+	+	+	-
Capric acid	+	-	-	+	+	+	-	-
Adipic acid	+	+	+	-	-	-	-	+
Malic acid	+	-	+	+	+	+	+	-
Trisodium citrate	+	+	-	-	+	+	+	-
Enzyme activities (API ZYM)								
Esterase Lipase (C8)	+	+	+	+	-	+	-	+
Lipase (C14)	+	-	+	-	-	-	+	+
Valline arylamidase	+	+	+	+	-	+	-	+
Cystine arylamidase	+	-	+	+	+	+	+	+
Trypsin	+	+	+	+	+	+	+	+
α-Chymotrypsin	-	-	-	+	+	+	+	-
Acid phosphatase	+	+	-	-	-	+	-	-
DNA G + C content (mol%)	62.1	63.8	59.8[a]	61.4[b]	59.5[c]	60.4[d]	61.3[e]	66.3[f]

[a] Dua *et al.* (2013); [b] Romanenko *et al.* (2013); [c] Yoo *et al.* (2006); [d] Lee (2007); [e] Verma *et al.* (2009); [f] Quan *et al.* (2020).

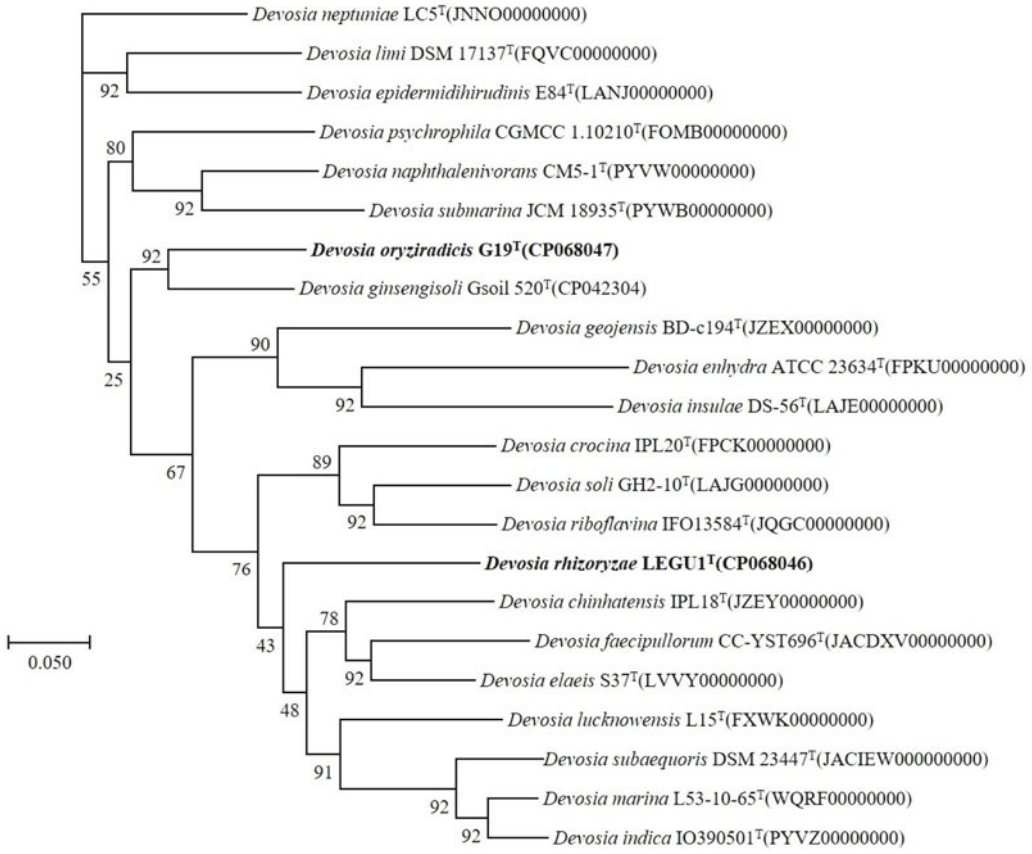

Fig. 2. Phylogenomic tree of strains LEGU1^T and G19^T and closely related strains based on core genomes was constructed using UBCG, genomes of all 20 related strains are available on NCBI GenBank. GenBank accession numbers are shown in parentheses. Bootstrap analysis was carried out using 100 replications. Percentage bootstrap values (> 50%) are given at branching points. Bar, 0.050 substitution per position.

comparison of clusters of genes between two novel strains is provided in Fig. 3. The result was also compared with the most closely related species of two novel strains (Fig. 3). The ANI and dDDH values between strain LEGU1^T and G19^T and their close relatives were < 81.9% and 25.1%, respectively (Table 1), these values were clearly lower than prokaryotic species delineation thresholds criteria (ANI, ~95%; *in silico* DDH, 70%) (Stackebrandt and Goebel, 1994; Chun *et al*., 2018), suggesting that strains LEGU1^T and G19^T represent novel species within the genus *Devosia*. Species belonging to the genus *Devosia* have been examined for their bioremediation potential and are known for their presence in habitats contaminated with various toxins, such as diesel-contaminated soils and hexa-chlorocyclohexane dump sites (Talwar *et al*., 2020). The genomes of strains LEGU1^T and G19^T were found to have gene clusters for bacterial toxin-antitoxin (TA) systems, which are key regulators of cellular processes that can respond to external stimuli and promote survival during periods of stress. A TA locus is composed of two genes coding for a toxin and its cognate antitoxin (Schuster and Bertram, 2013). Under favorable conditions, antitoxins typically inhibit their cognate toxins. However, under stressful conditions, antitoxins are readily proteolysed, thereby unleashing their inhibitory effects on toxin. Strain LEGU^T had more gene clusters for TA systems than strains G19^T (Supplementary data Table S1). Gene clusters for siderophore biosynthesis, auxin response and tryptophan biosynthesis were annotated in the genomes of both strains. Auxin producing bacteria are known to stimulate seed germination, root formation and root proliferation, thereby providing the host plant with greater access to water and soil nutrients (Glick, 1995). Siderophores are iron scavenging ligands that are produced by a wide range of rhizosphere bacteria to help solubilization and transport iron through the formation of soluble Fe^{3+} (Hopkinson and Barbeau, 2012).

Gene clusters for siderophore biosynthesis, auxin response and tryptophan biosynthesis were annotated in the genomes of two novel strains and their reference strains. The number of genes for auxin response and tryptophan biosynthesis were almost same in all species however gene clusters for siderophore biosynthesis were vary in all strains. Intrestingly, the novel strains LEGU1^T and G19^T contain less number of genes for siderophore as compared to their close relatives. Among all species, *D. crocina* KACC 14589^T, *D. riboflavina* KACC 11387^T, and *D. ginsengisoli* KACC 19440^T had the highest number of genes for siderophore. A comparison of genes responsible for IAA and siderophore biosynthesis between two novel strains is provided in Supplementary data Table S2. Cobalamin (Vitamin B12) has been suggested to stimulate plant development and could be synthesized either via *de novo*

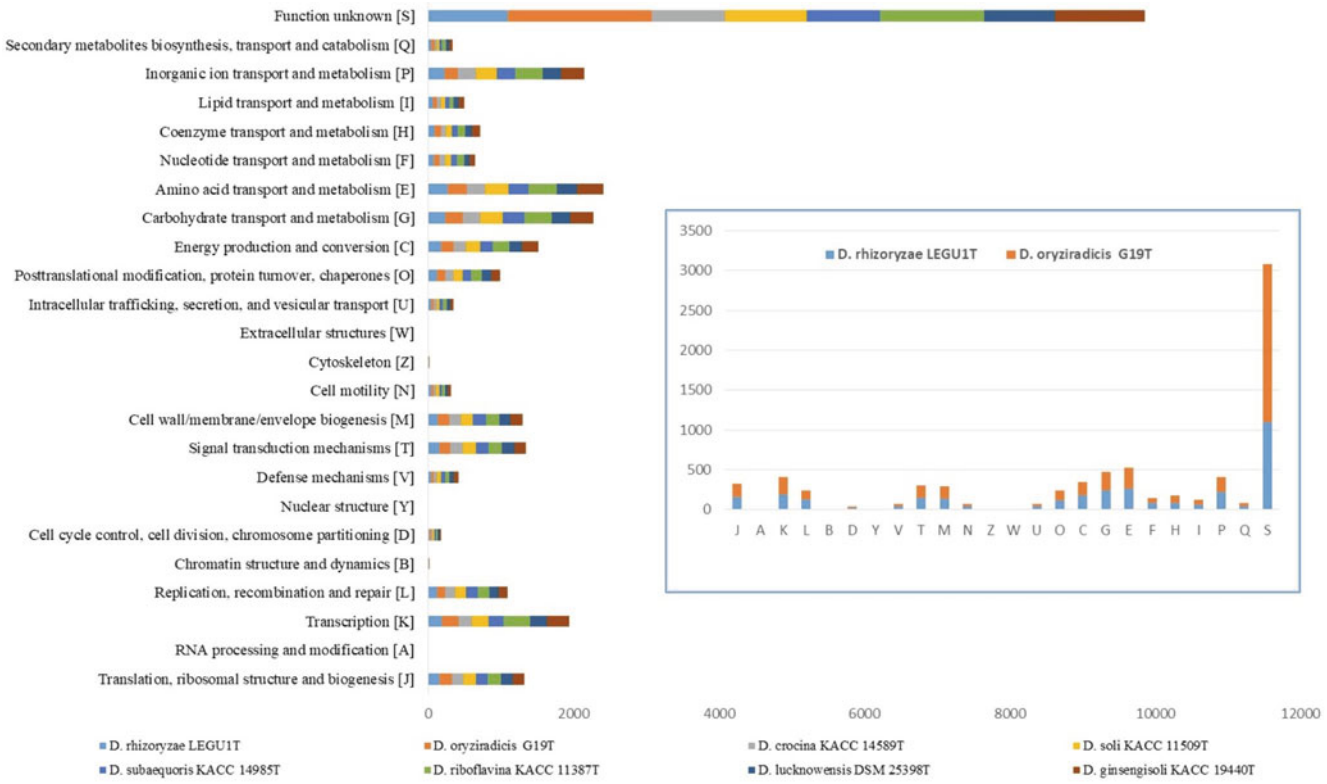

Fig. 3. Comparison of genes based on the 24 general eggNOG functional categories of strains LEGU1^T and G19^T and their reference strains.

or salvage pathways. Strain LEGU1^T contain three sets of gene clusters for cobalamin: cobalamin-independent methionine synthase II family protein, cobalamin biosynthesis protein and cobalamin biosynthesis protein (CobW) and strain G19^T contains two sets of gene clusters for co-balamin biosynthesis protein and cobalamin biosynthesis protein (CobW) in their genomes.

Chemotaxonomic characterization

Strains LEGU1^T and G19^T contained Q-10 as the sole quinone which is identical to other members of the genus *Devosia* (Quan *et al*., 2020). Moreover, both strains contained phosphatidylglycerol (PG) and diphosphatidylglycerol (DPG) as their major polar lipids. Although the major polar lipid profile showed a similar pattern, the minor polar lipid profile differed among the analyzed strains (Supplementary data Fig. S5). The additional lipids in strain LEGU1^T were three unidentified glycolipids (GL1-GL3) and three unidentified lipids (L1-L3). In Strain G19^T, one unidentified phospholipid (PL), three unidentified glycolipids (GL1-GL3) and four unidentified lipids (L1-L4) were the additional polar lipids. The presence and absence of minor lipids differentiates the two novel strains from each other and with their reference strains. We compared the polar lipid of strain LEGU1^T and G19^T with available data of *D. lucknowensis* DSM 25398^T and *D. ginsengisoli* KACC 19440^T, because they were most close to novel strains. Absence of unidentified phospholipid in strain LEGU1^T distinguish it from strain G19^T, *D. lucknowensis* DSM 25398^T and *D. ginsengisoli* KACC 19440^T. In addition, presence of three unidentified lipids in strain LEGU1^T and its absence in its most close strain *D. lucknowensis* DSM 25398^T clearly dis-

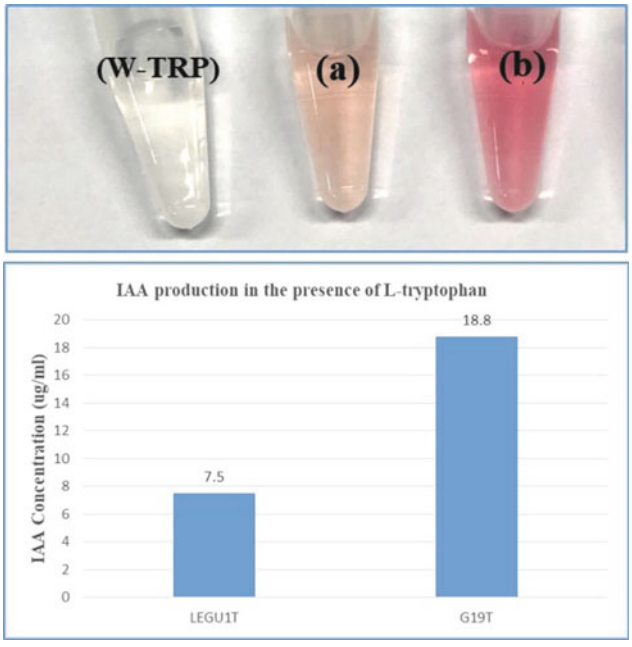

Fig. 4. Salkowski test for indole-3-acetic acid (IAA) produce by strains LEGU1^T (a) and G19^T (b). (a) and (b) are bacterial supernatant with Salkowski's reagent (In the absence of tryptophan). W-TRP, bacterial supernatant without Salkowski's reagent.

tinguish them. In strain, G19^T, presence of three unidentified glycolipids and four unidentified lipids and absence of three unidentified aminolipids distinguish it from strain *D. ginsengisoli* KACC 19440^T.

The major fatty acids of strain LEGU1^T and G19^T were $C_{16:0}$, $C_{18:1}$ *ω7c* 11-methyl and summed feature 8 (comprising $C_{18:1}$ *ω7c*/$C_{18:1}$ *ω6c*). The fatty acids profile of analysed strains were similar to the closest reference strains. Despite the comprehensive similarities, the amounts of major and minor fatty acids differed between the analyzed strains and their closest neighbours (Supplementary data Table S3). In case of strain LEGU1^T, presence of $C_{11:0}$, iso $C_{11:0}$, and absence of $C_{15:0}$ 2OH and $C_{18:1}$ *ω5c* differentiates it from strain G19^T and other close strains. In strain G19^T, absence of $C_{11:0}$, iso $C_{11:0}$ and presence of $C_{18:1}$ *ω5c* distinguish it from strain LEGU1^T and other close strains.

Quantification of IAA

PGPR are a group of free living soil bacteria, that can promote the growth and yield of crop plants through direct and indirect mechanisms. Phytohormones play an important role as signals and regulators of plant growth and development. Of these, auxin is the most studied plant growth regulator (Cleland *et al.*, 1995). Strains LEGU1^{T}and G19^T showed the ability to synthesize IAA in the presence of the precursor L-tryptophan and could produce 7.5 and 18.8 µg/ml IAA, respectively (Fig. 4). Both strains were also able to produce siderophores, this was confirmed by the production of uncolored halos around colonies on Chrome Azurol S (CAS) agar which is blue in color (data not shown).

Taken together, the findings of phylogenetic, physiological, biochemical, chemotaxonomic, and genomic analyses performed in the present study suggest that strains LEGU1^T and G19^T represent two novel species within the genus *Devosia*. Both strains were able to produce IAA and siderophore. These results suggest that both strains has good application potentials in sustainable agriculture. We propose the name *D. rhizoryzae* sp. nov. for strain LEGU1^T and the name *D. oryziradicis* sp. nov. for strain G19^T.

Description of *Devosia rhizoryzae* sp. nov.

Devosia rhizoryzae (rhiz.o.ry'zae. Gr. fem. n. *rhiza* root; L. fem. n. *oryza* rice; N.L. gen. n. *rhizoryzae* of rice roots).

Cells are Gram-staining-negative, strictly aerobic, motile by flagella and rod-shaped. Colonies are circular, smooth, convex and orange-pigmented with diameter of 0.8–1.6 µm after 5 days of incubation at 30°C on R2A agar. Growth occurs at 4–40°C (optimum 30°C), pH 6.0–9.0 (optimum 7) and in the presence of 0–4% (w/v) NaCl (optimum 0%). Cells are positive for catalase activity and negative for oxidase activitiy. Cells can produce carotenoid and flexirubin-type pigments. Cells grew well on R2A, moderate growth on LB, NA and TSA but do not grow on MA. Nitrate is not reduced to nitrite and glucose is not fermented. Cells are positive for production of indole and arginine dihydrolase. Cells are positive for the hydrolysis of urease, esculin and gelatin, however they are negative for the hydrolysis of CM cellulose, starch, casein and chitin. The assilimation of D-glucose, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid malic acid and trisodium citrate occurs however the assilimation of *β*-galactosidase, L-arabinose, D-mannose, D-mannitol and phenylacetic acid does not occur. In API ZYM, cells are positive for alkaline phosphatase, esterase, esterase lipase, lipase, leucine arylamidase, valline arylamidase, cystine arylamidase, trypsin, acid phosphatase, naphtol-AS-BI-phosphohydrolase, *β*-galactosidase, *α*-glucosidase, *β*-glucosidase, and N-acetyl-*β*-glucosaminidase but negative for *α*-chymotrypsin, *α*-galactosidase, *β*-glucuronidase, *α*-mannosidase and *α*-fucosidase. The major fatty acids are $C_{16:0}$, $C_{18:1}$ *ω7c* 11-methyl and summed feature 8 (comprising $C_{18:1}$ *ω7c*/$C_{18:1}$ *ω6c*). Q-10 is the predominant respiratory quinone. The polar lipids are phosphatidylglycerol, diphosphatidylglycerol, three unidentified glycolipids and three unidentified lipids.

The type strain, isolated from roots of rice plants collected from a paddy field in Ilsan, Republic of Korea, is LEGU1^T (= KCTC 82712^T = NBRC 114485^T). The G + C content of the genomic DNA is 62.1 mol%.

Description of *Devosia oryziradicis* sp. nov.

Devosia oryziradicis (o.ry.zi.ra′di.cis. L. fem. n. *oryza* rice; L. fem. n. *radix-icis* root; N.L. gen. n. *oryziradicis* of the rice root).

Cells are Gram-stain negative, strictly aerobic, flagellated and rod-shaped. Colonies are smooth, convex, opaque, circular with regular margins, white in colour, 1–3 mm in diameter after 3 days of incubation at 30°C on R2A agar. Growth occurs at 7–38°C (optimum, 30°C), pH 5.0–9.0 (optimum, 7.0) and in the presence of 0–5% (w/v) NaCl (optimum, 0%). Cells do not produce carotenoid and flexirubin-type pigments. Cells grew well on R2A, moderately on LB, TSA, NA, and MA. Nitrate is not reduced. Cells are positive for indole production, glucose fermentation, arginine dihydrolase production. Hydrolysis of urease occurs however, the hydrolysis of esculin, gelatin, casein, CM-cellulose, starch and chitin does not occur. The assimilation of D-glucose, L-arabinose, D-mannose, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, adipic acid and trisodium occurs, however, the assimilation of *β*-galactosidase, capric acid, malic acid and phenylacetic acid does not occur. In API ZYM, cells are positive for alkaline phosphatase, esterase, esterase lipase, leucine arylamidase, cystine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, *α*-glucosidase, *β*-galactosidase and N-acetyl-*β*-glucosaminidase but negative for lipase, cystine arylamidase, *α*-chymotrypsin, *α*-galactosidase, *β*-glucuronidase, *α*-mannosidase and *α*-fucosidase. The major fatty acids are $C_{16:0}$, $C_{18:1}$ *ω7c* 11-methyl and summed feature 8 (comprising $C_{18:1}$ *ω7c*/$C_{18:1}$ *ω6c*). Q-10 is the predominant respiratory quinone and the polar lipids are phosphatidylglycerol, diphosphatidylglycerol, one unidentified phospholipid, three unidentified glycolipids and four unidentified lipids.

The type strain, isolated from roots of rice plants collected from a paddy field in Goyang, South Korea, is G19^T (= KCTC 82688^T = NBRC 114842^T). The G + C content of the genomic DNA is 63.8 mol%.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Statements

This study does not describe any experimental work related to human.

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