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ORIGINAL PAPER

Devosia oryzisoli sp. nov., a novel moderately halotolerant bacterium isolated from the roots of rice plants and genome mining revealed the biosynthesis potential as plant growth promoter

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Abstract A Gram-stain-negative, halotolerant bacterium designated as PTR5^T was isolated from the roots of rice plants, collected in Ilsan, South Korea. Cells were, aerobic, asporogenous, motile, rod-shaped, white in color, and grew at 5–38 °C (optimum 30 °C), at pH 5.0-0-8.0 (optimum, 7.0) and tolerates up to 10% (w/v) NaCl (optimum, 0% NaCl). According to the EZbioCloud server the most closely related *Devosia* species to strain PTR5^T based on 16 S rRNA gene sequence comparison are *Devosia crocina* (97.4%), followed by *D. soli* (97.2%), *D. lucknowensis* (96.9%) and *D. marina* (96.5%). The respiratory quinone was identified as Q-10. The major polar lipids were phosphatidylglycerol and diphosphatidylglycerol. $C_{16:0}$, $C_{18:1}$ $\omega 7c$ 11-methyl and summed feature 8 (comprising $C_{18:1}$ $\omega 7c$/$C_{18:1}$ $\omega 6c$) constituted the main cellular fatty acids. The draft genome sequence of strain PTR5^T was 3,689,283 bp in size. The average nucleotide identity (ANI), digital DNA–DNA hybridization (dDDH) and amino acid identity (AAI) values between strain PTR5^T and its close relative were 72.8–76.8%, 19–20.7% and 70.3–75%, respectively. The G+C content was 63.7%. Strain PTR5^T was able to produce siderophore and indole acetic acid (IAA) in the presence of L-tryptophan. Genes for siderophore production, auxin responsive and tryptophan biosynthesis were present in the genome of novel strain. Also, gene clusters involved in detoxification of various metal pollutants and antibiotics were also revealed in the genome of novel strain PTR5^T, this suggest that novel strain can facilitate bioremediation of heavy metals and antibiotics in contaminated areas. This study aimed to determine the detailed taxonomic position of the strain PTR5^T using the modern polyphasic approach. On the basis of evidence presented in this study, strain PTR5^T is considered to represent a novel species of the genus *Devosia*, for which the name *Devosia oryzisoli* sp. nov. (type strain PTR5^T (KCTC 82691^T = TBRC 15163^T) is proposed.

Keywords *Devosia* · Indole acetic acid · L-Tryptophan · Exopolysaccharide · Novel · Genome

Abbreviations

ANI	Average nucleotide identity
dDDH	Digital DNA–DNA hybridization
AAI	Amino acid identity
PGP	Plant growth-promoting
IAA	Indole acetic acid
KCTC	Korean Collection for Type Cultures

Repositories The draft genome and 16 S rRNA gene sequences of strain PTR5^T have been deposited at GenBank/EMBL/DDBJ under accession numbers JACYFU000000000 and OP763491 respectively.

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TBRC Thailand Bioresource Research Center
EPS Extracellular polymeric substance
CAS Chrome Azurol S

Introduction

The genus *Devosia* has been created by the reclassification of *Pseudomonas riboflavina* as *Devosia riboflavina* (Nakagawa et al. 1996). Until April 2022, the genus *Devosia* comprises 32 species with validly published names″ (https://lpsn.dsmz.de/genus/devosia). Members of the genus are ubiquitous in diverse ecological niches including nitrifying inoculum (Vanparys et al. 2005), roots of rice plant (Chhetri et al. 2022), hexachlorocyclohexane dump sites (Dua et al. 2013), soil (Yoon et al. 2007), deep sediment (Jia et al. 2014), beach sediment (Lee et al. 2007), medical leech (Galatis et al. 2013) and alpine glacier (Zhang et al. 2012). Members of the genus *Devosia* are described as Gram-negative, flagellated, rod-shaped, obligately, contains Q-10 or Q-11 as the predominant respiratory quinone and have diverse colony colors like yellow, white, yellow-brown, orange and cream. Members of the genus *Devosia* are best studied for their potential to degrade several toxic compounds, establishing their promising candidature for bioremediation (Sato et al. 2012). Mycotoxins are fungal metabolites toxic to animals and can be accumulated in crop plants and creates a health risk for humans and livestock. Many toxins like fumonisins and trichothecenes are heat-stable and cannot be deactivated by cooking. Previous studies showed that many bacteria isolated for the degradation of deoxynivaleonol (DON) were identified as *Devosia* strains (Wang et al. 2019). In our recent study we isolated two members of genus *Devosia* found to produce siderophore and auxin (Chhetri et al. 2022). In the present study, we again isolated a novel species of genus *Devosia*, examined the amounts of IAA produced, detected the siderophore synthesis abilities and examined its taxonomic positions by a polyphasic approach including genome analysis. Genome sequencing allowed us to identify genes that might be involved in its plant growth-promoting (PGP) capacity. The annotated genome sequence of novel strain revealed vital gene clusters involved in exopolysaccharide synthesis, toxin-antitoxin system, and resistant against various antibiotics and metals, which are of considerable biotechnological value.

Isolation and cultivation

Strain PTR5^T was isolated from the sterilized roots of fresh rice plants in Ilsan, South Korea, (GPS positioning of the sample collection site; 37°40′26.4″ N 126°48′20.88″ E). The samples were washed with tap water, and then immersed in 70% alcohol for 3 min and washed 3 times with sterile water. The root samples were then ground in a sterile pottery mortar, which produced a suspension including bacteria from the rhizoplane and from the inner tissues of the roots. Subsequently, the suspension was diluted with double-distilled water using the standard dilution plating technique and then incubated at 28 °C on Reasoner's 2 agar (R2A; Difco) for one week (Kim et al. 2019a). A single colony was chosen based on different morphology from the plates, purified by repeated streaking and transferred to new R2A plates. Selected colonies were sent to Bionics (Daejeon, Republic of Korea) for 16 S rRNA gene analysis. The isolate was preserved in 50% (v/v) glycerol at − 80 °C. Strain PTR5^T has been deposited in the Korean Collection for Type Cultures (KCTC, Korea) and Thailand Bioresource Research Center (TBRC).

16 S rRNA gene phylogeny

TaKaRa MiniBEST Bacteria Genomic DNA extraction Kit version 3.0 (TaKaRa) was used for extraction of genomic DNA in accordance with the manufacturer's instructions. The 16 S rRNA gene of the isolate was directly amplified by colony-PCR using four universal bacterial primers 27 F, 518 F, 805R and 1492R; PCR products were commercially sequenced (Solgent, Korea). The almost complete sequence (1354 nt) of the 16 S rRNA gene of strain PTR5^T determined in this study was compared, using the CLUSTALX 2.1 programme (Thompson et al. 1997), with those of representatives of the genus *Devosia* and multiple sequences were aligned using MEGA 7.0 software (Kumar et al. 2016). Phylogenetic analyses were analysed with neighbor-joining (NJ) (Saitou and Nei 1987), maximum-likelihood (ML) (Felsenstein 1981) and

maximum-parsimony (MP) (Fitch 1971). Evolutionary distances were calculated using Kimura's two-parameter model (Kimura 1980) and the robustness of the topology in the phylogenetic trees was evaluated by bootstrap analyses based on 1000 resamplings (Felsenstein 1985).

Genomic features

The draft genome of strain PTR5^T was sequenced using the Illumina HiSeq platform by Macrogen Co., Ltd. (Seoul, Republic of Korea) and assembled using the SOAPdenovo version 3.10.1 de novo assembler. For genome sequencing of strain PTR5^T, a standard DNA library was prepared using the TruSeq DNA PCR-Free kit library (Illumina). Barrnap (0.9-dev) (https://github.com/tseemann/barrnap) was used for further validation of the 16 S rRNA gene (1454nt) in the genome, and both results yielded identical results. The genomic DNA G+C content was determined directly from the draft genome sequence. The ANI, dDDH and AAI values between the strain PTR5^T and closely related members were analysed using the online webservers (www.ezbiocloud.net/tools/ani) (Yoon et al. 2017), (http://ggdc.dsmz.de) (Meier-Kolthoff et al. 2013) and (http://enve-omics.ce.gatech.edu/aai/), respectively. For the AAI values, protein sequences were predicted from genomic sequences using GeneMarksS. The CheckM bioinformatics tool was used to assess genome contamination and completeness (https://ecogenomics.github.io/CheckM) of strain PTR5^T (Parks et al. 2015). The function of coding genes in the assembled genome were annotated by using server database called evolutionary genealogy of genes: Nonsupervised Orthologus Groups (eggnog) 4.5 (Huerta-Cepas et al. 2016). Whole genome sequences of close strains were downloaded from NCBI and a phylogenomic tree based on the concatenation of 92 core genes was constructed by using UBCG (Na et al. 2018). Genes involved in secondary metabolism were predicted by antibiotics and Secondary Metabolite analysis shell (antiSMASH) version 5.0 (Blin et al. 2019). Genome annotation was conducted by the NCBI prokaryotic genome annotation pipeline (PGAP) (Tatusova et al. 2016) and Rapid Annotation using Subsystem Technology (RAST) server (Aziz et al. 2008).

Physiology and chemotaxonomy

Based on 16 S rRNA gene sequence similarities and phylogenetic trees analysis results, *D. crocina* KACC 14,589^T, *D. soli* KACC 11,509^T, *D. subaequoris* KACC 14,985^T, *D. lucknowensis* DSM 25,398 and *D. riboflavina* KACC 11,387^T were selected as reference strains and were evaluated together with strain PTR5^T under identical experimental conditions. The phylogenetically related type strains, *D. soli* KACC 11,509^T, *D. crocina* KACC 14,589^T, *D. subaequoris* KACC 14,985^T, *D. riboflavina* KACC 11,387^T and *D. lucknowensis* DSM 25,398 were purchased from Korean Agricultural Culture Collection (KACC) and Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSM) and used as reference strains for physiological tests and fatty acid detection. Cell morphology of strain PTR5^T was observed on R2A agar after 48 h of incubation at 30 °C. Cells were observed under transmission electron microscope (TEM) (LIBRA 120, Carl Zeiss, Germany), using cells grown in R2A at 30 °C. For TEM preparation, the cells were suspended in distilled water and a grid was placed on the suspension for one minute, followed by negative staining of the cells with phosphotungustic acid (PTA). Gram staining was performed according to the procedure described previously (Kim et al. 2019b). Motility was evaluated in R2A medium containing 0.4% agar. Growth under anaerobic conditions was observed by incubating the cells in a GasPak jar (BBL, Cockeysville, MD, USA) at 30 °C for 10 days. Catalase activity was determined by bubble formation in 3% (v/v) H_2O_2, while oxidase activity was analysed by oxidation of 1% (w/v) tetramethyl-p-phenylenediamine (bioMérieux). Tolerance of the cells to various NaCl concentrations was tested in R2A broth containing 0–10% NaCl (w/v, at 0.5% intervals) as described previously (Chhetri et al. 2020). Growth at different temperatures (3, 5, 10, 18, 30, 35, 38, 42, and 45 °C) and various pH values (pH 4.5–11.0 at intervals of 0.5 pH units) was assessed after 7 days of incubation. Growth of strain PTR5^T was monitored for 7 days on R2A agar under different temperatures (3, 5, 10, 18, 30, 35, 38, 42, and 45 °C). The pH range was assessed by cultivating cells in R2A broth adjusted to pH 4.0–10.0 (at 1.0 pH unit intervals) using citrate/NaH2PO4 buffer (pH 4.0–5.0), phosphate buffer (pH 6.0–8.0), Tris buffer (pH 9.0–10.0) and Sodium phosphate buffer (pH 11.0) at 30 °C for 7 days. Growth was evaluated at 30 °C on several standard bacteriological

media: R2A agar, MA (marine agar), trypticase soy agar (TSA), nutrient agar (NA) and Luria Bertani agar (LB; all from Difco). The presence of flexirubin-type pigments was investigated using 20% (w/v) KOH solution (Kim et al. 2020). Extraction of cells for carotenoid analysis were achieved with a 10 ml methanol/acetone mixture (1:1, v/v) and the absorption spectrum of the pigments was assessed with a spectrophotometer (Multiskan GO; Thermo Fisher Scientific) (So et al. 2022).

Hydrolysis of chitin, CM cellulose, starch, and casein was determined as previously described (Kim et al. 2019a). Hydrolysis of Tween 80, Tween 60 and Tween 40 was examined as described by Smibert and Krieg (1994). Additional biochemical tests for strain PTR5^T and its reference strains were performed using API ZYM and API 20 NE kits according to the methods described by the manufacturer (bioMérieux) under equivalent test conditions.

For cellular fatty acid analysis, cells of strain PTR5^T and other reference strains were grown on R2A medium broth at 30 °C and were collected at late-exponential phase. Cells were extracted by saponification, methylation and extraction, as reported previously (Kuykendall et al. 1988). The Sherlock Microbial Identification System V6.01 (MIS, database TSBA6, MIDI Inc., Newark, DE, USA) was used to identify the extract.

Respiratory quinone was extracted with chloroform/methanol (2:1, v/v), evaporated under a vacuum, re-extracted with acetone and analyzed using high-performance lipid chromatography (HPLC) in accordance with the protocol described in previous reports (Collins and Jones 1981).

The polar lipids were extracted as described previously (Minnikin et al. 1984) and 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 were used to identify the spots (Komagata and Suzuki 1987; Kang et al. 2021).

Indole acetic acid (IAA), siderophore production and nitrogen fixation

Strains PTR5^T was grown in R2A broth with or without 0.1% tryptophan for 5 days at 30 °C. After 5 days of incubation the cells were centrifuged at 6000 rpm for 30 min. 1ml of supernatant was transferred and mixed with 2ml of Salkowski's reagent (2% 0.5 M, $FeCl_3$ in 35% $HClO_4$ solution), and incubated at room temperature for 30 min, and spectrophotometrically assessed at 530 nm. IAA production was quantified by using a standard curve with known concentrations of pure commercial IAA. Uninoculated R2A broth was treated as a negative control. Change in pink color indicated the production of IAA. The results were compared with and without L-tryptophan as described previously (Chhetri et al. 2022). Strain PTR5^T was examined for siderophore production on Chrome Azurol S (CAS) plates. Strain PTR5^T was spot inoculated and incubated at 30 °C for 5 days. Since the strain PTR5^T was isolated from the roots of rice plants its nitrification ability was also assessed. Jensen's nitrogen free medium was used for this purpose, and bromothymol blue (BTB) was used as an indicator. Growth of strain PTR5^T in nitrogen free medium was observed for one week. All experimental analyses were performed in triplicate to ensure reproducibility.

Results and discussion

16 S rRNA gene sequence similarities and phylogenetic analysis

The 16 S rRNA gene sequence analysis in the EzBioCloud database showed that, strain PTR5^T was closest to *D. crocina* KACC 14,589^T (97.4%), followed by *D. soli* KACC 11,509^T (97.2%), *D. subaequoris* KACC 14,985^T (97.2%) and *D. lucknowensis* DSM 25,398 (96.2%), *D. submarina* KMM 9415^T (96.5%) and *D. riboflavina* KACC 11,387^T (96.4%). The 16 S rRNA sequence similarities among the strain PTR5^T and other species of the genus were 94.0-97.4%. In neighbor-joining tree, strain PTR5^T formed an independent lineage within the genus *Devosia* close to *D. soli* KACC 11,509^T, *D. crocina* KACC 14,589^T, *D. riboflavina* KACC 11,387^T and *D. salina* (Fig. S1). The 16 S rRNA gene phylogenetic trees obtained by the ML and MP algorithms consistently showed identical topologies (Fig. S2 and Fig. S3).

Genomic features

The draft genome of strain PTR5^T was composed of 13 contigs and the total genome size was 3.69 Mb with a DNA G+C content of 63.7 mol%. The numbers of coding genes and tRNA genes were 3472 and 46, respectively. The assembly process found no evidence for plasmids and the final sequence yielded was deposited in NCBI GenBank under the accession number JACYFU010000000. The ANI value between strain PTR5^T and its close relatives calculated by using the EzBioCloud web server were 75.1–78%, which was far below the 95–96% threshold for the description of novel species (Yoon et al. 2017). The estimated dDDH value of strain PTR5^T with its close relatives of genus *Devosia* were 19–21.1%, which was far lower than the threshold value of 70% for the definition of bacterial species (Meier-Kolthoff et al. 2013) (Table 1). In the phylogenomic tree, strain PTR5^T formed an independent lineage close to its reference strains which is consistent with other phylogenetic trees (Fig. 1). Since the core gene phylogeny showed that the genus *Devosia* is polyphyletic, therefore the AAI values between strain PTR5^T and type strains of neighboring genera were also calculated. The AAI values were in the range of 60.3–63.9%,

Table 1 Comparative genomic features with average nucleotide identity, digital DNA–DNA hybridization and AAI values among strain PTR5^T and closely related members

Strains	Accession number	Genome size (bp)	G+C content (%)	PTR5^T		
				OrthoANIu %	dDDH %	AAI %
Devosia oryzisoli PTR5^T	JACYFU000000000	3,689,283	63.7	–	–	
Devosia crocina IPL20^T	FPCK00000000	3,723,990	61.3	75.8	20.3	73.6
Devosia soli GH2-10^T	LAJG00000000	4,136,371	61	76.1	20.7	74.6
Devosia subaequoris DSM 23,447^T	JACIEW000000000	3,785,142	61.1	76.1	19.9	75
Devosia lucknowensis L15	FXWK00000000	3,719,665	62.9	76.8	20.4	75
Devosia submarina JCM 18,935^T	PYWB00000000	3,980,379	60.4	74.5	19.7	71.6
Devosia riboflavina IFO13584^T	JQGC00000000	5,052,234	61.8	76.5	20.9	73.6
Devosia epidermidihirudinis E84^T	LANJ00000000	3,859,784	61.1	74.6	19.7	70.5
Devosia elaeis S37^T	LVVY00000000	3,878,148	64.1	78	21.1	75.9
Devosia psychrophila CGMCC 1.10210^T	FOMB00000000	4,328,275	61.2	74.9	19.5	71.2
Devosia limi DSM 17,137^T	FQVC00000000	4,297,227	62.7	75.1	19.7	70.7
Devosia indica IO390501^T	PYVZ00000000	3,953,285	61.9	76.7	20.6	71.3
Devosia naphthalenivorans CM5-1^T	PYVW00000000	4,487,701	61.4	75.1	19.8	71.6
Devosia chinhatensis IPL18^T	JZEY00000000	3,497,719	62.4	76.5	20	75.5
Devosia insulae DS-56^T	LAJE00000000	5,750,119	65.3	73.1	19	70.3
Devosia enhydra ATCC 23,634^T	FPKU00000000	4,220,684	65.6	72.8	19.3	72.3
Devosia geojensis BD-c194^T	JZEX00000000	4,465,063	65.9	75.2	20	70.6
Devosia marina L53-10-65^T	WQRF00000000	3,868,180	61.3	74.5	20.4	75.2
Devosia ginsengsoli Gsoil 520^T	CP042304	4,480,314	63.7	76.2	20.6	72.2
Devosia faecipullorum CC-YST696^T	JACDXV000000000	3,400,074	62.2	76.8	20.7	74.4
Arsenicitalea aurantiaca 42-50^T	RZNJ00000000	3,686,775	65.4	72.3	18.6	63.3
Youhaiella tibetensis CGMCC 1.12719^T	BMFM00000000	4,425,532	64.8	73.7	19.1	63.9
Paradevosia shaoguanensis J5-3^T	CP068983	4,629,545	63.9	73.7	19.2	65.3
Pelagibacterium luteolum CGMCC 1.10267^T	FNCS00000000	4,296,808	60.4	71	20.2	60.4
Pelagibacterium halotolerant H642^T	FNRH00000000	3,940,580	61.4	71.3	18.7	60.5
Pelagibacterium lacus XYN52^T	QQNH00000000	3,272,393	64.5	72.1	19	60.5
Pelagibacterium lentulum CGMCC 1.15896^T	BMKB00000000	4,042,346	57.7	72.1	18.8	60.3

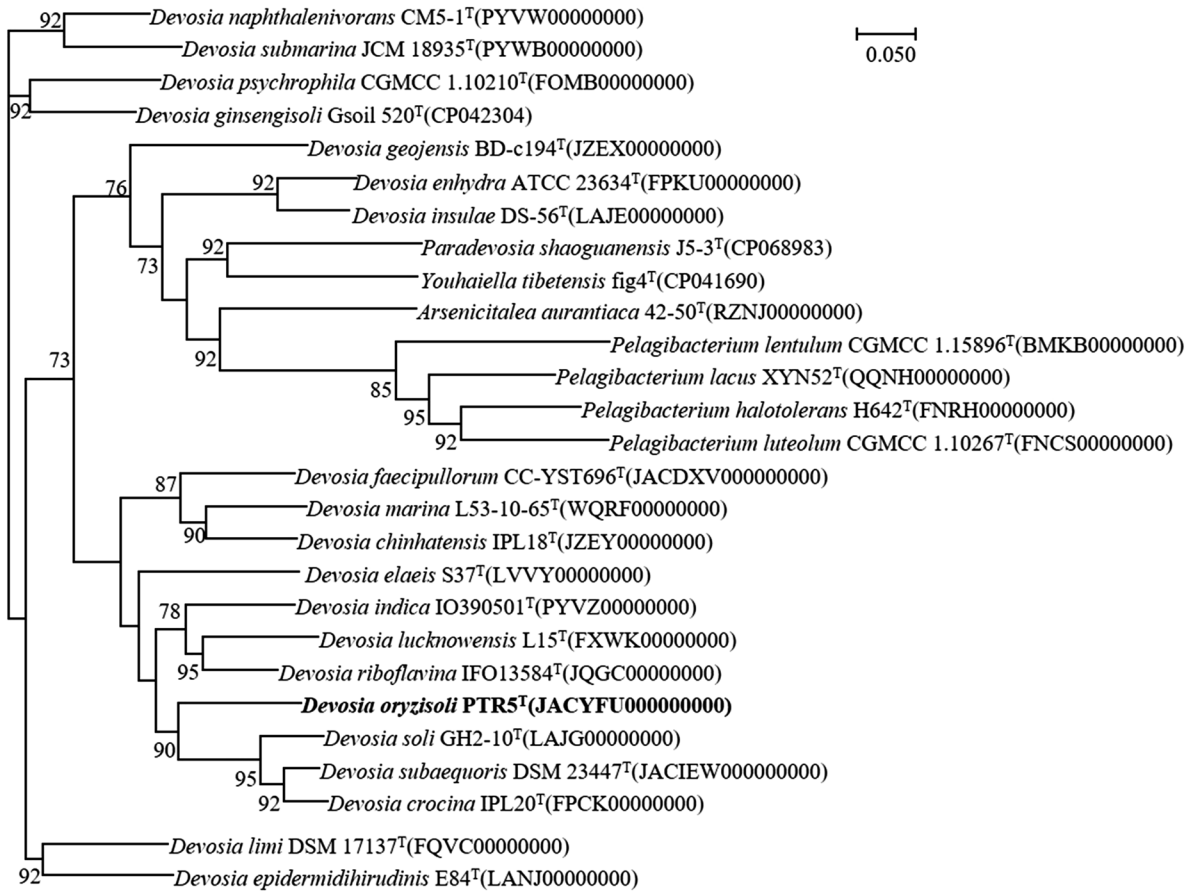

Fig. 1 Phylogenomic tree of strain PTR5^T and closely related strains based on core genomes was constructed using UBCG, genomes of all 26 related strains are available on NCBI GenBank. GenBank accession numbers are shown in parentheses. Bootstrap analysis was carried out using 100 replications. Percentage bootstrap values (> 50%) are given at branching points. Bar, 0.050 substitution per position

which is far below the threshold value for genus delimitation (65%). CheckM results revealed a 98.9% genome completeness and an estimated 0% genome contamination. Clusters of orthologous genes data of the strain PTR5^T genome dataset, obtained from eggNOG analysis, denoted that a total of 1075 genes were assigned to 24 functional categories. Among the obtained functional groups, the cluster for [E] (amino acid transport and metabolism; 279), [G] (carbohydrate transport and metabolism; 265), [P] (inorganic ion transport and metabolism; 235), [K] (transcription; 205), [C] (Energy production and conversion; 174), [J] (translation, ribosomal structure and biogenesis; 162 genes), [T] (signal transduction mechanisms; 163), were the most highly represented categories (in descending order). The comparison of clusters of genes between strain PTR5^T and its reference strains is provided in Fig. 2.

Genome analysis with antiSMASH revealed six gene clusters namely two homoserine lactone biosynthetic gene cluster, one betalactone biosynthetic gene cluster, one terpene biosynthetic gene cluster, one Type III PKSs (T3PKS) biosynthetic gene cluster and one ectoine biosynthetic gene cluster which was 83% similar to ectoine. Presence of ectoine biosynthetic gene clusters is responsible for halotolerant characteristics of strain PTR5^T. Biosynthetic gene clusters that are responsible for different secondary metabolites in strain PTR5^T and its reference strains were compared. Genes for terpene and hserlactone (homoserine lactone) were present in all strains. T3PKS gene cluster was only present in strain PTR5^T. Genes for ectoine

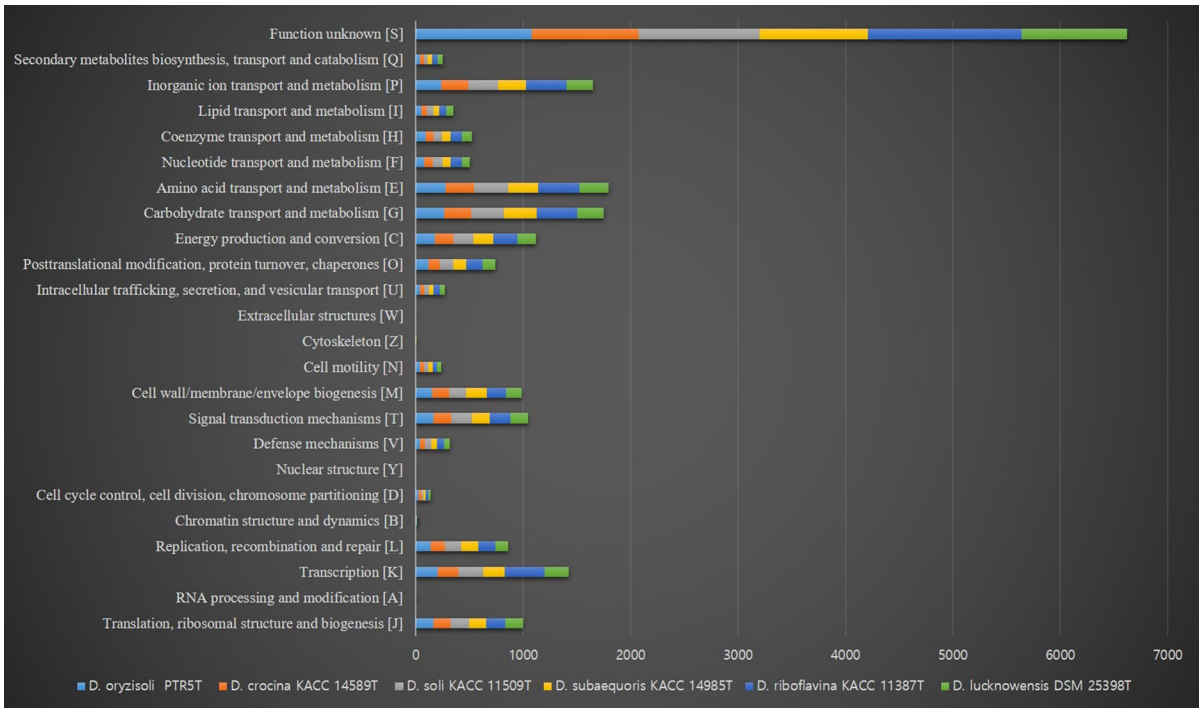

Fig. 2 Comparison of genes based on the 24 general eggNOG functional categories of strain PTR5^T with its phylogenetically related species of the genus *Devosia*

was present in all strains except *D. riboflavina* KACC 11,387^T, *D. soli* KACC 11,509^T. The presence and absence of gene clusters is presented in table S1.

NCBI annotation revealed six proteins for ectoine biosynthesis: ectoine/hydroxyectoine ABC transporter ATP-binding protein EhuA (JACYFU010000004), ectoine/hydroxyectoine ABC transporter permease subunit EhuD (JACYFU010000004), ectoine/hydroxyectoine ABC transporter permease subunit EhuC (JACYFU010000004), ectoine/hydroxyectoine ABC transporter substrate-binding protein EhuB (JACYFU010000004), ectoine hydroxylase (JACYFU010000004) and ectoine synthase (JACYFU010000004). It can be used as a protective agent for enzymes against stress conditions such as heat, cold, and high or low pH (Galinski and Trüper 1994; Kunte et al. 2014). NCBI also revealed two gene clusters for exopolysaccharide: exopolysaccharide biosynthesis polyprenyl glycosylphosphotransferase (JACYFU010000004), exopolysaccharide biosynthesis protein (JACYFU010000001). Eight gene clusters for polysaccharide proteins were also revealed in the genomes of strain PTR5^T, namely one polysaccharide biosynthesis C-terminal domain-containing protein (JACYFU010000001), one divergent polysaccharide deacetylase family protein (JACYFU010000003), two polysaccharide deacetylase family protein (JACYFU010000001), one polysaccharide export protein (JACYFU010000001), one polysaccharide biosynthesis/export family protein (JACYFU010000002) and one polysaccharide biosynthesis protein (JACYFU010000002) (Table S1). PTR5^T produces sufficient amount of extracellular polymeric substances (EPS) on agar plates, the presence of genes and the phenotypic results were consistent with each other. Gene clusters for siderophore biosynthesis, auxin response and tryptophan biosynthesis were annotated in the genome strain PTR5^T and its reference strains. The number of genes for auxin response and tryptophan biosynthesis were almost same in all species however number of gene clusters for siderophore biosynthesis were vary in all strains. Interestingly, the novel strain PTR5^T contain less number of genes for siderophore as compared to other close relatives. Among all species, *D. crocina* KACC 14,589^T and *D. riboflavina* KACC 11,387^T had the

highest number of genes for siderophore. Genes related to nitrogen fixation were not found. Presence of genes responsible for IAA and siderophore biosynthesis among strains is provided in Table S1. Candidate genes involved in resistance to antibiotics and toxic compounds were also investigated using RAST Server. In strain PTR5^T, 39 gene clusters were found including: copper homeostasis (16), cobalt-zinc-cadmium resistance (11), mercuric reductase (1), tetracycline resistance, ribosome protection type (4), copper tolerance (3), resistance to fluoroquinolones (2), beta-lactamase (1) and resistance to chromium compounds (1). Its reference strains also contains genes involved in resistance to antibiotics and toxic compounds, the comparison of number of genes is presented in table S2. The heavy metals As, Au, Zn, Cd, Ur, Se, Ag, Hg, Cr and Ni are hazardous heavy metals that contaminate the environment and adversely affects the quality of the soil, crop production as well as public health (Kuhlmann et al. 2011; Ndeddy Aka et al. 2016; Glick 1995). The application of strain PTR5^T, as bioremediation of heavy metals and antibiotics contaminated soils can enhance plant growth against heavy metal toxicity and increase heavy metal removal efficiency.

Physiology and chemotaxonomy

Cells of strain PTR5^T were Gram-reaction-negative, motile, aerobic, non-spore-forming and long-rods. Morphology of cells of strain PTR5^T is available as Fig. 3. Colonies were white-pigmented, and smooth and watery after incubation for 3 days on R2A agar. Strain PTR5^T could grow on R2A, LB, NA, MA and TSA agar. Growth occured at 5–38 °C (optimum, 30 °C), pH 4.0–8.0 (optimum, pH 7.0) and 0–10% (w/v) NaCl (optimum, 0%). Cell growth was not found in the addition of 10.5% NaCl. PTR5^T produces sufficient amount of EPS on agar plates. EPS provide a microenvironment that holds water and dries more slowly compared with the surrounding environment, thus protecting bacteria and roots of plants against desiccation (Sandhya et al. 2009). The hydrolysis of urease, esculin, casein, CM-cellulose, starch and chitin does not occur while that of gelatin occurs. Other reference strains did not produced EPS in agar plates which differentiates the strain PTR5^T visually from its close relatives. Absence of pigmentation in colonies of strain PTR5^T differentiates it with its most close relatives. Moreover, the higher NaCl tolerance ability of strain PTR5^T differentiates it from its reference strains. Other phenotypic features of strain PTR5^T were summarized in the species description and properties differentiating the isolate from type strains of closely related species were detailed in Table 2.

The major fatty acids (> 10% of the total fatty acids) detected in strain PTR5^T were $C_{16:0}$, $C_{18:1}$ $\omega 7c$ 11-methyl and summed feature 8 (comprising $C_{18:1}$ $\omega 7c/C_{18:1}$ $\omega 6c$) in line with the reference strains. The little percentage of $C_{11:0}$ and absence of $C_{16:0}$ in strain PTR5^T differentiate it from other close relatives. Differences between strain PTR5^T and the reference strains were detailed in Table S3. The predominant respiratory quinone detected in strain PTR5^T was ubiquinone Q-10 and the major polar lipids were phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), one unidentified phosphoglycolipid (PGL), two unidentified aminolipids (AL1-2), three unidentified aminoglycolipids (AGL1-3), two unidentified glycolipids (GL1-2) and two unidentified lipids (L1-2), which were consistent with other members of the genus *Devosia* (Fig. S4).

Plant growth promoting traits

Strains PTR5^T showed the ability to synthesize IAA in the presence of the precursor L-tryptophan and could produce 19.4 µg/ml IAA (Fig. S6). Growth of strain PTR5^T was not observed in Jensen's nitrogen free medium which confirm that this novel strain is not able to fix nitrogen like most species of *Devosia*. Strain PTR5^T was also able to produce siderophores, as this was confirmed by the production of uncolored halos around colonies on CAS agar which is blue in color (not shown).

Description of *Devosia oryzisoli* sp. nov.

Devosia oryzisoli (o.ry.zi.so'li. L. fem. n. *oryza* rice; L. gen. n. *soli* of the soil; N.L. gen. n. oryzisoli of the rice root).

Cells are Gram-stain negative, aerobic, flagellated and rod-shaped. Colonies are smooth, convex, opaque, circular with regular margins, white in color, and 1–3 mm in diameter and produce slime materials after 3 days of incubation at 30 °C. Growth occurs

Table 2 Physiological and biochemical characteristics of strain PTR5^T and closely related type strains of the genus *Devosia*. Strain: 1. PTR5^T; 2 *D. crocina* KACC 14,589^T; 3, *D. soli* KACC 11,509^T; 4, *D. subaequoris* KACC 14,985^T; 5, *D. lucknowensis* DSM 25,398; 6, *D. riboflavina* KACC 11,387^T. Data are from this study unless indicated. +, Positive; −, negative

Characteristics	1	2	3	4	5	6
Isolation source	Plant	HCN dump site	Green house soil	Deep sea surface	HCN contaminated pond soil	Soil
Colony colour	White	Orange	Light beige	Orange-red	Orange	Cream
Cell shape	Rod	Oval	Rod	Rod	Rod	Rod
Catalase/oxidase	+/+	+/−	+/−	+/+	+/+	+/+
Temperature range for growth (°C)	5–38	5–32	10–37	5–38	5–37	5–38
NaCl range for growth	0–10	0–3	0–5	0–5	0–3	0–3
pH range for growth	5.0–8.0	5.0–9.0	4.0–8.0	6.0–9.0	6.0–9.0	5.0–9.0
Media for optimum growth	R2A, LB	LB, TSA	R2A	TSA, MA	R2A	R2A, TSA
EPS production	+	−	−	−	−	−
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	−	−	−	+	−	−

at 5–38 °C (optimum, 30 °C), pH 4.0–8.0 (optimum, 7.0) and in the presence of 0–10% (w/v) NaCl (optimum, 0%). Cells do not produce carotenoid and flexirubin-type pigments. Cells grew well on R2A and LB and moderately on TSA, NA and MA. Cells are positive for catalase and oxidase activities. The hydrolysis of urease, esculin, casein, CM-cellulose, starch and chitin does not occur while that of gelatin occurs. In API 20NE, strain showed positive results for indole production, glucose fermentation and arginine dihydrolase production. The assimilation of $_L$-arabinose, $_D$-mannose, $_D$-mannitol, $_D$-maltose and potassium

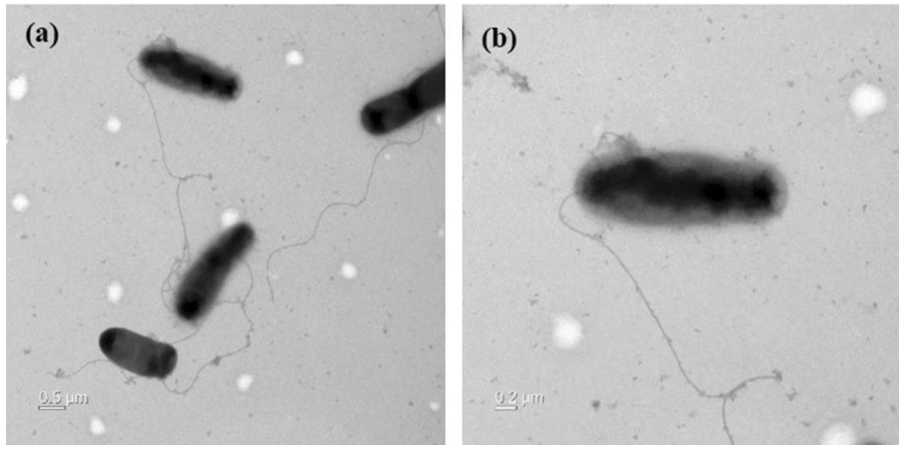

Fig. 3 Transmission electron microscopy of strain PTR5^T. Cells were negatively stained with phosphotungstic acid after growth at 30 °C on R2A agar for four days. Bar (**a**) and (**b**) 0.5 µm and 0.2 µm bar

gluconate occurs In API ZYM, cells are positive for alkaline phosphatase, esterase, esterase lipase, leucine arylamidase, valline arylamidase, naphtol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, β-glucosidase and $_N$-acetyl-β-glucosaminidase. The major fatty acids are $C_{16:0}$, $C_{18:1}$ $\omega 7c$ 11-methyl and summed feature 8 (comprising $C_{18:1}$ $\omega 7c$/$C_{18:1}$ $\omega 6c$). Q-10 is the predominant respiratory quinone and the polar lipids are phosphatidylglycerol, diphosphatidylglycerol, one unidentified phosphoglycolipid, three unidentified aminoglycolipids, two unidentified aminolipids, two unidentified glycolipids and two unidentified lipids.

The type strain, isolated from roots of rice plants collected from a paddy field in Ilsan, Republic of Korea, is PTR5^T (=KCTC 82691^T=TBRC 15163^T). The DNA G+C content of the strain is 63.7%. The GenBank/EMBL/DDBJ/PIR accession number for the 16 S rRNA gene sequence of strain PTR5^T is OP763491. The NCBI accession number for the whole genome sequence of strain PTR5^T is JACYFU000000000.

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Declarations

Conflict of interest The authors declare that there is no conflict of interest.

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

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