**Oryzicola mucosus** gen. nov., sp. nov., a novel slime producing bacterium belonging to the family **Phyllobacteriaceae** isolated from the rhizosphere of rice plants

Geeta Chhetri · Jiyoun Kim · Inhyup Kim · Minchung Kang · Yoonseop So · Taegun Seo

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**Abstract** A novel Gram-stain negative, asporogenous, slimy, rod-shaped, non-motile bacterium ROOL2T was isolated from the root samples collected from a rice field located in Ilsan, South Korea. Phylogenetic analysis of the 16S rRNA sequence showed 96.5% similarity to **Tianweitania sediminis** Z8T followed by species of genera **Mesorhizobium** (96.4–95.6%), **Aquabacterium** (95.9–95.7%), **Rhizobium** (95.8%) and **Ochrobactrum** (95.6%). Strain ROOL2T grew optimally at 30 °C in the presence of 1–6% (w/v) NaCl and at pH 7.5. The major respiratory quinone was ubiquinone-10 and the major cellular fatty acids were C18:1ω7c, summed feature 4 (comprising iso-C17:1 I and/or anteiso-C17:1 B) and summed feature 8 (comprising C18:1ω6c and/or C18:1ω7c). The polar lipid profile consisted of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, phosphatidylmethylethanolamine, phosphatidylglycerol, one unidentified aminolipid and two unidentified lipids. The assembled draft genome of strain ROOL2T had 28 contigs with N50 value of 656,326 nt, total length of 4,894,583 bp and a DNA G+C content of 61.5%. The average amino acid identity (AAI) values of strain ROOL2T against the genomes of related members belonging to the same family were below 68% and the ANI and dDDH values between the strain ROOL2T and the type strains of phylogenetically related species were 61.8–76.3% and 19.4–21.1%, respectively. Strain ROOL2T only produces carotenoid-type pigment when grown on LB agar and slime on R2A agar. In the presence of tryptophan, strain ROOL2T produced indole acetic acid (IAA), a phytohormone in plant growth and development. Gene clusters for indole-3-glycerol phosphatase and tryptophan synthase were found in the genome of strain ROOL2T. The genotypic and phenotypic characteristics indicated that strain ROOL2T represents a novel genus belonging to the family **Phyllobacteriaceae**, for which the name **Oryzicola mucosus** gen. nov., sp. nov. is proposed. The type strain is ROOL2T (KCTC 82711 T = NBRC 114717 T).

**Keywords** Oryzicola mucosus · Phytohormone · Indole acetic acid · Slime · Carotenoid

**Abbreviations**
ANI Average amino acid identity
IAA Indole acetic acid
KCTC Korean Collection for Type Cultures
NBRC Biological resource Centre, NITE
MEGA Molecular Evolutionary Genetics Analysis
NJ Neighbour-joining
ML Maximum-likelihood
MP Maximum-parsimony
UBCG Up-to-date bacterial core gene
DPG Diphosphatidylglycerol
PE Phosphatidylethanolamine

Introduction

The family Phyllobacteriaceae belongs to the order Rhizobiales, class Alphaproteobacteria of phylum Proteobacteria and was originally described by Knösel (1984). At the time of writing, the family consisted of twenty genera (https://lpsn.dsmz.de/family/phyllobacteriaceae). Of these Aquamicrobium species use nitrate as an alternative terminal electron acceptor, while Mesorhizobium species are facultatively chemolithotrophic and can use thiosulfate or elemental sulfur as the sole energy source. Most of the members of Mesorhizobium and Phyllobacterium have plant growth promoting effects (Willems et al. 2014). Some members may be involved in the degradation of hazardous pollutants (Maynaud et al. 2013; Mahieu et al. 2011). Over 80% of rhizosphere bacteria may be capable of synthesizing IAA, a plant growth hormone. The bacterial cells belonging to the family Phyllobacteriaceae are usually Gram-stain negative, rod-shaped, aerobic, non-spore forming and non-motile or motile by means of polar, subpolar or lateral flagella. Ubiquinone-10 (Q-10) is the predominant isoprenoid quinone. While assessing the bacterial biodiversity in rice plants, strain ROOL2T was isolated from their roots. Bacterial exopolysaccharides are the main component of the biofilm glyocalyx, which has also been coined the slime layer. When fully hydrated, the glyocalyx is predominantly water and provides a certain degree of protection for its inhabitants against environmental threats, including biocides, antibiotics, antibody, surfactants, bacteriophages, and foraging predators such as free-living amoebae (Brading et al. 1995). The present study aimed to determine the taxonomic position of slime and IAA producing strain ROOL2T using a polyphasic approach, which included phenotypic, chemotaxonomic, phylogenetic and genomic analysis and together the results indicate that this strain represents a novel species belonging to a novel genus within the family Phyllobacteriaceae.

Materials and methods

Isolation of the novel strain, cultivation and morphological study

Root samples were collected from a paddy field near Ilsan, South Korea, (GPS positioning of the sample collection site; 37° 40’ 26.4” N 126° 48’ 20.88” E). The root samples were thoroughly washed with sterile water. The root was cut into small fragments and macerated using sterile pestle as described previously (Chhetri et al. 2020). The macerated samples were serially diluted using 0.85% NaCl. Isolation was performed on R2A agar (Difco) at 28°C for 1 week. A single slimy colony was chosen from the plates and purified by transferring to new R2A plates. The purified colonies were sent to Bionics (Daejeon, Republic of Korea) for 16S rRNA gene analysis. From the purified bacterial colonies, a novel strain belonging to the genus was identified to be a member of the family Phyllobacteriaceae and was designated as ROOL2T. For routine work, cells were stored in R2A broth containing 50% (v/v) glycerol at −80°C. Strain ROOL2T was deposited in the Korean Agricultural Culture Collection (KCTC 82711 T) and Biological resource Centre, NITE (NBRC 113689 T). Cell morphology, size and absence of flagella were observed by transmission electron microscope (TEM) (LIBRA 120, Carl Zeiss, Germany), using cells grown in R2A at 30°C. A grid was placed on the suspension for a minute and followed by negative staining using phosphotungstic acid (PTA), washing twice with distilled water for 2 s and drying for 3 min.

Phylogenetic analysis based on 16S rRNA gene sequence

Genomic DNA extraction and PCR amplification of the 16S rRNA gene of strain ROOL2T were performed
as described previously (Chhetri et al. 2019). The sequence was compared with 16S rRNA gene sequences of valid species from the EzBioCloud server (https://www.ezbiocloud.net/) and GenBank using the BLAST program. Multiple sequences were aligned using MEGA 7.0 software (Kumar et al. 2016) and analyzed using the CLUSTALX 2.1 (Thompson et al. 1997). Distance matrices were calculated according to Kimura’s two parameter model. Phylogenetic trees were reconstructed using the neighbour-joining, maximum-likelihood and maximum parsimony methods (Saitou and Nei 1987; Felsentein et al. 1981; Kluge and Farris 1969). The distances were calculated using Kimura’s two-parameter distance model. Bootstrap values were determined on the basis of 1000 replications.

**Physiological and biochemical characteristics**

Growth in the presence of different NaCl concentrations, at different pH values and at different temperatures was assessed as described previously (Kim et al. 2019b,a). The growth of the strain on the following media was assessed at 30 °C for 5 days: R2A agar (Difco), trypticase soy agar (TSA; Difco), marine agar (MA; Difco), nutrient agar (NA; Difco) and Luria–Bertani agar (LB; Difco). Anaerobic growth was assessed by checking for colony formation on R2A agar at 30 °C for 10 days in a GasPak jar (BBL, Cockeysville, MD, USA), while motility was assessed in TSA medium containing 0.4% agar. Gram reaction was determined using the non-staining KOH lysis method (Buck et al. 1982). Catalase activity was determined as the production of bubbles after the addition of 3% (v/v) hydrogen peroxide (H₂O₂). Oxidase activity was determined by employing 1% (w/v) tetramethyl-p-phenylenediamine. Moreover, gliding motility was tested using the hanging-drop method after growing the cells in R2A broth (Difco) for 48 h at 30 °C (Bernardet et al. 2002). Hydrolysis of chitin, carboxymethyl-cellulose, starch, and casein was also evaluated according to a previously described method (Smibert et al. 1994). Additional enzyme activities, biochemical features and physiological characteristics were tested using the API 20NE and API ZYM kits (bioMérieux) according to the manufacturer’s instructions.

**Indole acetic acid (IAA) production**

Strain ROOL2ᵀ was grown in LB medium with or without 0.1% tryptophan at 30 °C for 3 days. Cell culture was centrifuged at 6000 rpm for 30 min after 3 days of incubation. Supernatant was reserved and 1 ml was mixed with 2 ml of Salkowski’s reagent (2% 0.5 FeCl₃ in 35% HClO₄ solution), then incubated at room temperature for 30 min. Indole production was indicated by color change into pink. Result was compared with and without tryptophan.

**Chemotaxonomy**

For chemotaxonomic analyses, strain ROOL2ᵀ and its reference strains were grown under same conditions on R2A medium at 30 °C. The cell mass was harvested after 4 days at the late-exponential phase. Cellular fatty acids of strain ROOL2ᵀ and its reference strains were obtained by saponification, methylation and extraction using previously reported method (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. Sep-Pak Vac cartridges (Waters Associates Inc., Milford MA USA) were used for the purification of isoprenoid quinones, and the extract was analyzed by high-performance lipid chromatography as reported previously (Hiraishi et al. 1996; Collins and Jones 1981).

Polar lipids were extracted as described previously (Minnikin et al. 1984). The mobile phases used for the first and second dimensions were chloroform/methanol/water (65:25:4, by vol.) and chloroform/methanol/acetic acid/water (80:12:15:4, by vol.), respectively (Minnikin et al. 1984). The specific functional groups of phosphates, sugars and amino-lipids were detected using molybdenum blue, α-naphthol and ninhydrin, respectively.

The presence of flexirubin-type pigments was investigated with 20% (w/v) KOH solution (Kim et al. 2019b,a). Hydrolysis of chitin, CM cellulose, starch, and casein was determined as previously described The extraction of cells for carotenoid analysis was performed using a 10 ml methanol/acetone mixture (1:1, v/v) and the absorption spectrum of the pigments was assessed with a
spectrophotometer (Multiskan GO, thermos Fisher Scientific) (Chhetri et al. 2021).

Genome assembly and annotation

For genome sequencing, a standard DNA library was prepared using the TruSeq DNA PCR-Free Library Prep Kit (Illumina). Subsequently, whole genome sequencing was performed by de novo sequencing analysis using an Illumina Hiseq 4000 sequencer with a paired-end read length of 151 bp and assembled using SPAdes Analysis v.3.10.1 at Macrogen (Republic of Korea). As the whole genome of Tianweitania sediminis Z8T was not available, the process was completed using the same procedure as that used for strain ROOL2^T. ANI values were calculated using an ANI calculator (Yoon et al. 2017). The average amino acid identity (AAI) was determined using the AAI calculator available online at Kostaslab with the default parameters (http://enve-omics.ce.gatech.edu/). Protein sequences were predicted from genome sequences using GeneMarksS (Besemer et al. 2001). Two-way AAI was calculated. Digital DDH (dDDH) is another method that allows in silico comparisons between two genomes (Meier-Kolthoff et al. 2013; Goris et al. 2007; Richter et al. 2009). The dDDH values were calculated between the genome sequence of strain ROOL2^T and the available reference genome sequences of its closest relatives. The values were estimated via GGDC version 2.1 using the recommended formula 2 (http://ggdc.dsmz.de/distcalc2.php), considering a cut-off value of 70%. The DNA G + C content of strain ROOL2^T was calculated on the basis of the genome data. In order to strengthen the phylogenetic status and better characterize the relationships between the strain ROOL2^T and its other closely related species, phylogenomic trees were constructed based on the basis of an up-to-date bacterial core gene set (UBCG) consisting of 92 core gene sequences (Na et al. 2018). Publicly available genomes of the closely related taxa were used. Genes involved in secondary metabolism were predicted using antibiotics and Secondary Metabolite analysis shell (antiSMASH) version 5.0 (Blin et al. 2019). Genome annotation was performed via the NCBI prokaryotic genome annotation pipeline (PGAP) and Rapid Annotation using Subsystem Technology (RAST) server (Aziz et al. 2008).

Results and discussion

Morphology, physiology and biochemical analysis

Strain ROOL2^T was isolated from the roots of rice plant, collected in South Korea. Cells were Gram-stain-negative, rod-shaped with no flagella (Fig. 1), non-spore forming and catalase and oxidase negative. Colonies of strain ROOL2^T were white to yellow, circular, convex and round, displayed slimy and mucoid appearance on R2A agar plates after 3–4 days of cultivation at 30 °C (Fig. S1). Some bacteria produce slime to adhere and float as colonial masses in their environments, thereby forming a protected environment for themselves (Wold P-A et al. 2014). On Luria–Bertani agar the strain appeared as yellow colonies and produced a carotenoid-type pigments, however no slime material was observed on LB agar. On R2A agar the strain made white colonies and produces slime materials. The absorption spectrum of the pigments extracted from the yellow colonies exhibited the characteristic profile of carotenoids with three absorption peaks at 426 nm, 451 nm and 481 nm (Fig. S1). Growth occurred at 20–35 °C (optimum 30 °C), and at pH 5.5–8.5 (optimum 7.5). Strain ROOL2^T did not require NaCl but tolerated up to 6.0% (w/v) NaCl with an optimum at 0%. Moreover, strain ROOL2^T was not able to hydrolyze chitin, casein, starch and CM-cellulose. In API 20NE kit, nitrate reduction and hydrolysis of esculin did occur. The morphological, physiological and biochemical characteristics of strain ROOL2^T exhibited a numerous phenotypic differences from closely related members e.g., production of watery slimy materials, production of carotenoid-type pigments only on LB agar, its growth range, the NaCl concentration tolerated for growth and its inability to assimilate almost all substrates in 20NE kit (Table 1). In addition, in API ZYM, the ability to show positive reactions for alkaline phosphatase and leucine arylamidase differentiated strain ROOL2^T from other reference members. Strain ROOL2^T was only negative for oxidase activity. Moreover, it could hydrolyze esculin, while the other two reference strains could not. Strain ROOL2^T was unable to ferment glucose and could not assimilate adipic acid. More phenotypic and biochemical characteristics that differentiate the novel strain ROOL2^T from its closely related members are presented in Table 1. The lack of motility of strain
ROOL2^T and the absence of motility and chemotaxis genes are further the main differences from the other members within the family Phyllobacteriaceae (Table S1).

Chemotaxonomic characterisation

The major fatty acids detected in strain ROOL2^T were C_{18:1}ω7c and summed feature 4 (comprising iso-C_{17:1} I and/or anteiso-C_{17:1} B) and summed feature 8 (comprising C_{18:1}ω6c and/or C_{18:1}ω7c) which differed from those detected the reference strains (Table S2). The presence of C_{18:1}ω7c as a major fatty acid in strain ROOL2^T and its absence in the closest reference strain T. sediminis Z8^T is the major difference between two strains. In addition, the absence of other minor fatty acids such as C_{16:0} and iso-C_{15:0} 3OH in strain ROOL2^T distinguishes it from T. sediminis Z8^T. Similar to other members belonging to the family Phyllobacteriaceae, Q-10 was the predominant isoprenoid quinone in strain ROOL2^T. The polar lipid profile of strain ROOL2^T consisted of diphasphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, phosphatidylmethylethanolamine, phosphatidylglycerol, one unidentified aminolipid and two unidentified lipids (Fig S2). The major polar lipid profile such as diphasphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine and phosphatidylmethylethanolamine were similar to other reference strains however the presence of other minor lipids could distinguish the strain ROOL2^T from other close members.

Phylogenetic, core gene phylogeny analyses and genomic features

Phylogenetic analysis of the almost complete 16S rRNA gene sequence of strain ROOL2^T (1413 bp) revealed that the strain belonged to the phylum ‘Proteobacteria’, in which it formed a distinct phyletic lineage within the family Phyllobacteriaceae and did not belong to any existing genera (Fig. 2). The 16S rRNA gene sequence similarity indicated that strain ROOL2^T was most closely related to T. sediminis Z8^T (96.5%) followed by M. tamadayense DSM 28320^T (96.4%), M. mediterraneum USDA 3392^T (96.2%) and A. soli NK8^T (96.1%), however, phylogenetic analysis based on 16S rRNA gene sequences suggested that strain ROOL2^T did not belong to any existing genera but formed a distinct phylogetic lineage within the family Phyllobacteriaceae in the neighbour-joining tree (NJ) (Fig. 2). Furthermore, phylogenetic trees including those reconstructed by maximum-likelihood (ML) (Fig. S3), maximum-parsimony (MP) (Fig. S4) and phylogenomic tree algorithms (Fig. S5), showed similar results in which strain ROOL2^T makes a separate lineage, which clearly indicating that strain ROOL2^T is a novel member within the family Phyllobacteriaceae at genus level. In all phylogenetic trees, M. tamadayense DSM 28320^T and M. mediterraneum USDA 3392^T were very far from strain ROOL2^T and formed a different cluster, and therefore in the present study, we considered T. sediminis Z8^T and A. soli NK8^T as reference strains for comparative analysis. However, as the
genomes of \textit{M. tamadayense} DSM 28320 \textsuperscript{T} and \textit{M. mediterraneum} USDA 3392 \textsuperscript{T} are publicly available, we compared these strains with strain ROOL2 \textsuperscript{T} on the basis of the genomic database and found that they clearly differ from each other. The 16S rRNA gene sequence of strain ROOL2\textsuperscript{T} has been deposited at GenBank/EMBL/DDBJ under accession the number MN904860.

The genome size of strain ROOL2\textsuperscript{T} was 4.89 Mb; it consisted of 28 contigs with N50 and L50 values of 656,326 and 3, respectively. The genome was found to have a coverage of 151.0x. The DNA G + C content was 61.5\%, this value is within the range reported for members belonging to the family \textit{Phyllobacteriaceae}. The calculated ANI, AAI and dDDH values between strain ROOL2\textsuperscript{T} and the closely related members \textit{T. sediminis} Z8\textsuperscript{T} and \textit{M. tamadayense} DSM 28320 \textsuperscript{T} are shown in Table 2. The phylogenomic tree based on the concatenated alignment of 92 core genes provided further evidence regarding the independent lineage of strain ROOL2\textsuperscript{T} (Fig. S5) confirming the 16S rRNA gene sequence analysis. Strain ROOL2\textsuperscript{T} shared 72.9 and 76.5\% ANI, 19.4 and 21.3\% dDDH, 64.3 and 67.7\% AAI values with \textit{T. sediminis} Z8\textsuperscript{T} (96.5\% 16S

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**Table 1** Physiological and biochemical characteristics of strain ROOL2\textsuperscript{T} and reference strains of the closely related species

| Characteristics                        | 1    | 2    | 3    |
|----------------------------------------|------|------|------|
| Colony color                           | White| White| Yellow|
| Motility                               | –    | +    | –    |
| Catalase/oxidase                        | –/–  | –/+  | +/+  |
| Growth at 37\textdegree C              | –    | –    | +    |
| NaCl range for growth                  | 0–6  | 0–3  | 0–4  |
| pH range for growth                    | 5.0–9.0| 6.0–8.0| 6.0–9.0|
| **Enzyme activities (API 20NE)**       |      |      |      |
| Nitrate reduction                      | +    | –    | +    |
| Arginine dihydrolase                   | –    | –    | +    |
| Esculin hydrolysis                     | +    | –    | –    |
| D–glucose                              | –    | +    | +    |
| L–arabinose                            | –    | –    | +    |
| D–mannose                              | –    | –    | +    |
| N–acetyl–D–glucosamine                 | –    | –    | +    |
| D–maltose                              | –    | –    | +    |
| Adipic acid                            | –    | +    | +    |
| Trisodium citrate                      | –    | +    | –    |
| Phenylacetic acid                      | –    | +    | –    |
| **Enzyme activities (API ZYM)**         |      |      |      |
| Alkaline phosphatase                   | +    | –    | –    |
| Esterase Lipase (C8)                   | +    | –    | +    |
| Lipase (C14)                           | –    | –    | +    |
| Leucine aryiamidase                    | +    | –    | –    |
| Cystine aryiamidase                    | –    | –    | +    |
| Trypsin                                | +    | –    | +    |
| \(\alpha\)–Chymotrypsin                | –    | –    | +    |
| Naphtol–AS–BI–phosphohydrolase         | +    | +    | –    |
| \(\alpha\)–Galactosidase              | –    | +    | –    |
| \(\alpha\)–Glucosidase                | +    | –    | –    |
| \(\beta\)–Glucosidase                 | –    | +    | +    |
| \(\alpha\)–Mannosidase                | –    | +    | –    |
| DNA G+C content (mol\%)                | 61.5 | 61.8 | 65.5 |

Strains: 1, ROOL2\textsuperscript{T}; 2, \textit{Tianweitania sediminis} Z8\textsuperscript{T}; 3, \textit{Aquamicrobium soli} NK8\textsuperscript{T}. All data were examined in this study unless otherwise indicated. +, Positive; –, negative; G + C content value of \textit{Aquamicrobium soli} NK8\textsuperscript{T} is from the original literature (Xu et al. 2017)
rRNA gene sequence similarity) and *M. tamadayense DSM 28320^T* (96.4% 16S rRNA gene sequence similarity), respectively, these values are far below the thresholds (95–96% for ANI, 70% for dDDH and 6.80% for AAI) proposed for bacterial species demarcation (Meier-Kolthoff et al. 2013; Goris et al. 2007; Richter et al. 2009; Qin et al. 2014). In the past, AAI values of 60–80% were considered thresholds for distinguishing genera however, recent studies on new genus descriptions within the family *Comamonadaceae* and other phylum have proposed that the threshold for genus boundaries should be 70% for AAI.
All the AAI values were found to be lower than the proposed genus boundary threshold, the ANI and dDDH values clearly indicated that strain ROOL2T is a novel member within the family Phyllobacteriaceae (Table 2). The phylogenomic tree constructed on the basis of the concatenated alignment of 92 core genes also confirmed the finding (Fig. S5).

The result of antiSMASH revealed that strain ROOL2T contains six biosynthetic gene clusters (BGCs) in its genome including bacteriocin, NAGGN, betalactone, arylpolyene, terpene and homoserine lactone (hserlactone) with a similarity value of 63, 28, 78, 61, 90 and 69%, respectively. RAST analysis revealed the presence of 322 subsystems and five secondary metabolism gene clusters for auxin biosynthesis. Four striking gene clusters for the synthesis of tryptophan (Trp) were found: kynA (tryptophan 2,3-dioxygenase; NZ_JACVVX010000003), trpB (tryptophan synthase subunit beta; NZ_JACVVX010000002.1), trpS (tryptophan–tRNA ligase, NZ_JACVVX010000001.1) and trpC (indole-3-glycerol phosphate synthase; NZ_JACVVX010000005.1). In addition one gene cluster for indole-3-glycerol phosphate synthase TrpC (JACVVX010000005) was also found. Bacterial IAA excretion increases root surface area and length, and thereby provides the plant greater access to soil nutrients and water uptake. Trp is the main precursor for IAA and auxin biosynthesis in microorganisms (Sessitsch et al. 2004). As the novel strain ROOL2T was isolated from the roots of rice plants, it can be directly or indirectly responsible for promoting the growth of rice plants. In addition our experimental results also showed the production of IAA by strain ROOL2T in the presence of tryptophan.

Strain ROOL2T can produce IAA in the absence of L-tryptophan also but in lower concentration. Strain ROOL2T made more IAA when L-tryptophan (Fig. S6) was added as precursor.

From this study, it is clear that rhizospheric strain ROOL2T has the ability to produce a significant amount of IAA in a tryptophan-supplemented medium. Isolation of IAA producing bacteria prevent environmental pollution by avoiding excessive applications of industrially produced fertilizers to cultivated fields. Moreover, the low values of ANI, dDDH and AAI indicated that, strain ROOL2T represents a distinct species within this novel genus. Based on the aforementioned findings, we propose that strain ROOL2T represents a novel genus in the family Phyllobacteriaceae, for which the name Oryzicola mucosus gen. nov., sp. nov. is proposed.

Description of Oryzicola gen. nov.

Oryzicola gen. nov. (O.ry.zi’co.la. L. n. oryza, rice; L. suff -cola, inhabitant, dweller; N.L. masc. n. Oryzicola, an inhabitant of rice).

Cells are Gram-stain negative, aerobic, asporogenous, rod shaped, non-flagellated, non-motile, catalase and oxidase negative. The bacterium reduces nitrate to nitrite. It contains Q-10 as the sole respiratory quinone. The major polar lipid profile consists of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, phosphatidyl-methylethanolamine and phosphatidylglycerol. The DNA G + C content of the type strain belonging to the type species is 63.5%. Based on phylogenetic analysis, the genus belongs to the family Phyllobacteriaceae within the phylum Proteobacteria. The type species is Oryzicola mucosus.

Description of Oryzicola mucosus sp. nov.

O. mucosus sp. nov. (mu.co’sus. L. masc. adj. mucosus, slimy)

Cells are Gram-stain negative, oxidase and catalase negative, non-motile and rod-shaped (0.8–1.0 µm length and 0.5–0.8 µm in width). Colonies on R2A agar are white coloured and produces watery slimy materials. However, colonies on LB agar are yellow and produced carotenoid-type pigments. They only produced carotenoid-type pigments on LB agar and slime material on R2A agar. IAA is produced in the presence of tryptophan. The optimum growth temperature is 28–30 °C; growth occurs at 20–35 °C. The bacterium shows good growth on R2A and LB agar and moderate growth on MA, TSA and NA. It does not hydrolyze casein, starch, carboxymethyl-cellulose, and chitin. It reduces nitrate. Flexirubin-type pigment is not produced. The strain is negative for indole production, glucose fermentation and hydrolysis of gelatin and β-Galactosidase. Assimilation of arginine dihydrolase, D-glucose, D-mannose, D-mannitol, D-maltose, L-arabinose, N-Acetyl-D-glucosamine,
potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid is not observed. Assessment of enzyme activities using the API ZYM kit revealed strain ROOL2T is positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, trypsin, acid phosphatase, napthol-AS-BI-phosphohydrolase, \( \alpha \)-Glucosidase and \( \beta \)-Glucosidase. However it is negative for lipase (C14), valline arylamidase, cystine arylamidase, \( \alpha \)-Chymotrypsin, \( \alpha \)-Galactosidase, \( \beta \)-Galactosidase, \( \beta \)-Glucuronidase, N-acetyl-\( \beta \)-Glucosaminidase, \( \alpha \)-Mannosidase and \( \alpha \)-Fucosidase. The major cellular fatty acids are C18:1\( \delta \)7c, summed feature 4 (comprising iso-C17:1 I and/or anteiso-C17:1 B) and summed feature 8 (comprising C18:1\( \omega \)6c and/or C18:1\( \omega \)7c). Q-10 is the predominant isoprenoid quinone. The polar lipid profile consist of diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylmethyl ethanolamine (PME), one unidentified aminolipid and two unidentified lipids. The DNA G + C content of the type strain is 61.5\%.

The type strain is ROOL2T (= KCTC 82711 T\(^\text{T} \) = NBRC 114717 T\(^\text{T} \)), which was isolated from the roots of rice plant. The draft genome and 16S rRNA gene sequences of strain ROOL2T have been deposited in GenBank/EMBL/DDBJ under accession numbers JACVVX0000000000 and MN9048600 respectively.

**Authors’ contributions** GC isolated the bacterium, designed the study, performed the phenotypic and biochemical characterization, and wrote the original draft; MK, JK, IK, YS helped with the analysis of taxonomic data; TS designed and supervised the study, and edited the original draft.

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**Declarations**

**Conflicts of interest** All the authors declare that there is no conflict of interest.

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

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