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Roseateles agri sp. nov., a New Species Isolated from Fresh Soil in Uiwang, South Korea

Yelim You and Jaisoo Kim *

Department of Life Science, College of Natural Sciences, Kyonggi University, Suwon 16227, Gyeonggi-do, Republic of Korea; nhanle@kyonggi.ac.kr
* Correspondence: jkimtamu@kgu.ac.kr; Tel.: +82-31-249-9648

Abstract: Two isolates of *Roseateles* were discovered in soil samples collected from Uiwang-si, Gyeonggi-do, Republic of Korea. These isolates exhibited rod-shaped morphology and were facultatively anaerobic, non-motile, and tested positive for oxidase and catalase. Designated as strains R3-3^T and R3-11, their growth was hindered by NaCl concentrations exceeding 0.5%, while their optimal growth conditions were observed at temperatures ranging from 25 °C to 30 °C and pH levels between 7.0 and 9.0. Both strains exhibited positive results for the hydrolysis of Tween 80 and DNA, but tested negative for starch, casein, chitin, and gelatin hydrolysis. Additionally, they assimilated L-Arabinose, D-mannitol, and D-Maltose, while exhibiting negative results for the fermentation of D-glucose, esculin ferric citrate, D-mannose, N-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. The DNA G+C content of strain R3-3^T was measured at 67.5 mol%. Comparative analysis revealed that the average nucleotide identity (ANI) values between R3-3^T and the *Roseateles* type strains ranged from 75.14% to 78.30% while the digital DNA-DNA hybridization (dDDH) values ranged from 20.70% to 22.70%. Consequently, based on comprehensive genomic, chemotaxonomic, phenotypic, and phylogenomic evaluations, the isolated strains have been designated as a new species within the genus *Roseateles*, named *Roseateles agri* sp. nov. (with type strain R3-3^T = KACC 23678^T = NBRC 116681^T).

Keywords: *Roseateles agri*; soil; new species; *Comamonadaceae*; *Burkholderiales*

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1. Introduction

The genus *Roseateles* belongs to the family *Comamonadaceae*, which is classified under the order *Burkholderiales* and the class *Betaproteobacteria*. It was initially proposed by Suyama et al. in 1999 [1]. Currently, there are 15 different species recognized within the genus *Roseateles*, with 14 validly published and correct names (https://lpsn.dsmz.de/genus/roseateles; date of access: 24 March 2024) [2]. Among these species, *R. depolymerans*, *R. aquatilis*, *R. terrae*, *R. oligotrophus*, *R. albus*, and *R. koreensis* were originally described as members of the genus Roseateles [2,3]. Additionally, the classifications of the genera *Pelomons*, *Kinneretia*, *Mitsuaria*, and *Paucibacter* were revised by Liu et al. in 2023 [3,4].

The phenotypic characteristics of *Roseateles* species are diverse. They are Gram-negative, non-endospore-forming, catalase-positive, rod-shaped bacteria. Some members of the genus are motile and possess flagella [1,5–7], while others are non-motile [8]. Additionally, some *Roseateles* species are facultatively anaerobic [7,8], whereas others are strictly aerobic [5,9]. Most known species of *Roseateles* are commonly found in water environments [1,3,5,7–11], although some have been isolated from sediment or mud [6,12], soil [13], and root nodules [14].

This study presents the characterization of strain R3-3^T, isolated from soil, and establishes its taxonomic position as a novel species within the genus *Roseateles*, proposed to be named *Roseateles agri* sp. nov.

2. Material and Methods

2.1. Isolation and Cultivation

In June 2023, a debris-free sieved fresh soil sample weighing 3 g was collected from the field soil at Cheongggye-dong, Uiwang-si, Gyeonggi-do, Korea (GPS coordinates: 37°24′52.6″ N, 127°02′29.4″ E). This sample was placed at the bottom of a six-transwell plate (Corning Inc., Corning, NY, USA) and supplemented with 3 mL of 50% R2A medium. Subsequently, 100 µL of the soil suspension was added to each insert, and the cultivation was carried out at 25 °C in a shaking incubator at 130 rpm for 2 weeks. Following the incubation period, the culture suspension was diluted, and 100 µL of each diluted culture was spread onto R2A agar plates, which were then cultivated at 28 °C for up to 5 days.

Individual colonies exhibiting distinct morphologies were selected and streaked onto R2A medium at 28 °C until pure colonies were obtained. These pure cultures were then preserved at −70 °C with 20% (*v*/*v*) glycerol until further analysis. All isolates retrieved from the plates underwent preliminary species identification via 16S sequencing. Among the isolates, two novel Roseateles strains (R3-3^T and R3-11) were identified, demonstrating potential as new species based on their 16S rRNA gene sequence identity.

Strain R3-3^T was deposited in the Korean Agriculture Collection (Seoul, Republic of Korea) and the National Institute of Technology and Evaluation (Osaki, Japan) under accession numbers KACC 23678 and NBRC 116681, respectively.

2.2. Physiology and Chemotaxonomy

The two strains were cultured on R2A (MBcell, Seoul, Republic of Korea) agar plates at 28 °C for 48 to 72 h, and colony morphology was observed visually. Cell morphology was scrutinized utilizing both light microscopy (BX50; Olympus, Tokyo, Japan) and transmission electron microscopy (Bio-TEM H-7650, Hitachi, Tokyo, Japan). Gram staining was carried out using Hucker's method [15], while assessment of endospore formation was conducted through malachite green staining as per the procedure outlined by Schaeffer and Fulton [16]. The standard incubation temperature was maintained at 28 °C unless specifically stated otherwise.

To determine oxidase activity, 1% (*w*/*v*) tetramethyl-p-phenylenediamine was utilized, while catalase activity was evaluated based on bubble formation upon mixing a pellet of fresh bacterial culture with a drop of 3% (*v*/*v*) hydrogen peroxide (H_2O_2). For identification of the optimal growth medium, various conventional media were tested, including R2A agar (MBcell), tryptone soya agar (TSA; MBcell), Luria Bertani agar (LB; MBcell), nutrient agar (NA; MBcell), Mueller–Hinton agar (MBcell), and MacConkey agar (MBcell).

Assessment of cell motility was conducted in R2A medium containing 0.4% agar. Growth at different temperatures (ranging from 4 °C to 40 °C) was monitored on R2A agar medium for 7 days. Salt tolerance tests were carried out in R2A broth with varying NaCl concentrations (ranging from 0.0% to 9.0% *w*/*v*) after 7 days of incubation.

The pH tolerance for growth was assessed by subjecting cells to R2A broth spanning pH values from 3.0 to 11.0 (at intervals of 0.5 pH unit), with different buffering agents used for each pH range. Examination of anaerobic growth was conducted on R2A agar plates enclosed in anaerobic bags (bioMérieux, Marcy-l'Étoile, France) at 28 °C over a period of 7 days.

Substrate hydrolysis trials, encompassing starch (Sigma-Aldrich, St. Louis, MO, USA), chitin (Tokyo Chemical Industry, Tokyo, Japan), casein (1% skimmed milk; MBcell), and Tween 80 (1%; Sigma-Aldrich), were executed on R2A agar medium for 7 days at 28 °C [17,18]. Methyl Red and Voges–Proskauer (MR-VP) assessments were performed utilizing MR-VP broth. API ZYM test strips (bioMérieux) were used to ascertain enzyme activity and acid production from sugars, while additional physiological and biochemical tests were carried out using API 20NE. Commercial kits API 20NE and API ZYM were used according to the manufacturer's instructions.

To investigate quinones and respiratory polar lipids, freeze-dried R3-3^T cells were cultured on R2A agar plates and harvested after two days of incubation. Quinones were

extracted from 100 mg freeze-dried cells of R3-3^T as described by Minnikin et al. (1984) [19] and analyzed with an Agilent 1260 infinity HPLC system. The extraction of polar lipids from R3-3^T was conducted following the methodology outlined by Minnikin et al. [19]. These lipids were then separated through two-dimensional thin-layer chromatography (TLC) using Merck silica gel 60 plates (10 cm × 10 cm). Following separation, specific detection reagents were employed: 5% (*w*/*v*) ethanolic molybdatophosphoric acid (Sigma-Aldrich) for total lipid profiling, a 0.4% (*w*/*v*) solution of ninhydrin (Sigma Life Science, St. Louis, MO, USA) in butanol for amino lipids, Zinzadze reagent (molybdenum blue spray reagent, 1.3%; Sigma Life Science) for phospholipids, and α-naphthol reagent (0.5%, *w*/*v*) for glycolipids.

For cellular fatty acid profiling, cultures of R3-3^T and R3-11 were cultivated on R2A agar medium at 28 °C for two days before harvesting. Cellular fatty acids were then extracted, saponified, and methylated following the protocol provided in the Sherlock Microbial Identification System version 6.3 (MIDI). Subsequently, gas chromatography (GC) was employed to analyze the fatty acid methyl esters, with identification conducted using the TSBA6 database of the Microbial Identification System [20].

2.3. 16S RNA Gene Phylogeny

The full 16S rRNA gene segment of strains R3-3^T and R3-11 underwent amplification employing the universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′). Subsequently, Macrogen (Seoul, Republic of Korea) conducted the sequencing of the DNA amplicons. To identify the most closely related type strain 16S rRNA gene sequences, the sequences obtained from R3-3^T and R3-11 were compared with entries in the EzBioCloud 16S rRNA gene sequence database (www.ezbiocloud.net/eztaxon, accessed on 10 March 2024) [21]. The 16S rRNA gene sequences of reference strains were retrieved from their respective entries in the NCBI GenBank database (www.ncbi.nlm.nih.gov/, accessed on 10 March 2024).

Alignment of the 16S rRNA gene sequences was carried out using the Silva alignment tool (www.arb-silva.de/aligner/, accessed on 10 March 2024). Phylogenetic analysis was performed using Mega 11 software, and trees were constructed utilizing three different methodologies: maximum-likelihood (ML), neighbor-joining (NJ), and minimum evolution (ME) methods. Bootstrap analyses, comprising 1000 resamplings, were conducted to determine bootstrap values, and evolutionary distances were computed using the Kimura two-parameter method [22].

2.4. Genome Features

The genomic DNA from strain R3-3^T was isolated utilizing DNeasy Blood and Tissue kits (Qiagen, Hilden, Germany). Macrogen (Seoul, Republic of Korea) conducted whole-genome shotgun sequencing of strain R3-3^T on an Illumina HiSeq platform, and subsequent sequence assembly was performed using SPAdes version 3.13.0.

To explore the evolutionary divergence based on whole-genome sequences, phylogenetic trees were generated for R3-3^T and 15 strains within the Roseateles genus, alongside some related strains in the *Comamonadaceae* family obtained from NCBI (www.ncbi.nlm.nih.gov/, accessed on 10 March 2024). The tree was computationally constructed using the concatenated alignment of 92 core genes through the UBCG pipeline [23].

Calculation of DNA G+C content and average nucleotide identity (ANI) values between the whole-genome sequences of R3-3^T and 15 strains within the Roseateles genus was carried out using the OrthoANIu algorithm [24]. Additionally, digital DNA–DNA hybridization (dDDH) values between R3-3^T and strains within the Roseateles genus were determined using the GGDC web server [25].

The genome sequences of R3-3^T were subjected to annotation using the Rapid Annotation with Subsystem Technology (RAST) server, version 2.0 [26]. To categorize genes based on their functions, cluster of orthologous group (COG) analyses were performed by querying the Kyoto Encyclopedia of Genes and Genomes (KEGG) database [27].

A Venn diagram illustrating the whole-genome orthologous genes was generated utilizing the Orthovenn3 web server [28] (https://orthovenn3.bioinfotoolkits.net/home, accessed on 20 March 2024).

3. Results and Discussion

3.1. Physiology and Chemotaxonomy

Colonies of both the strains grown on R2A agar were white, circular, convex, and glistening, with regular margins, and smooth surfaces, within 48–72 h at 28 °C (Figure 1). The colonies of R3-3^T and R3-11 on R2A agar were observed to have diameters ranging from 0.5 to 2 mm and 0.5 to 2.5 mm, respectively, after 72 h of incubation at 28 °C. Cell dimensions for all strains were within the range of 1.4–3.5 µm (length) and 0.5–1.0 µm (width). They were non-spore-forming, non-motile, rod-shaped, and stained Gram-negative (Figure S1). Growth was observed only on R2A agar, while there was no growth on TSA, LB, NA, Mueller–Hinton agar, and MacConkey agar. Positive oxidase and catalase activities were detected. Growth of both the strains was completely inhibited at NaCl concentrations above 0.5% (*w*/*v*). The pH range for growth of R3-3^T was 5.5–9.5, and for R3-11 it was 5.0–9.5, with the optimum pH for all strains being 7.0–9.0. The optimum temperature for growth ranged from 20 °C to 30 °C, with a growth range of 10 °C–35 °C. Both strains hydrolyzed Tween 80 but not casein, chitin, and gelatin. Phenotypic differences between the two strains and each of the closest known species with validly published names are detailed in Table 1. Notably, when considering only clearly positive or negative results, strains R3-3^T and R3-11 could be distinguished from the closest known species by their ability to assimilate L-Arabinose, D-Mannitol, and D-Maltose but not potassium gluconate (Table 1), as well as by their production of -galactosidase and -glucosidase (Table S1).

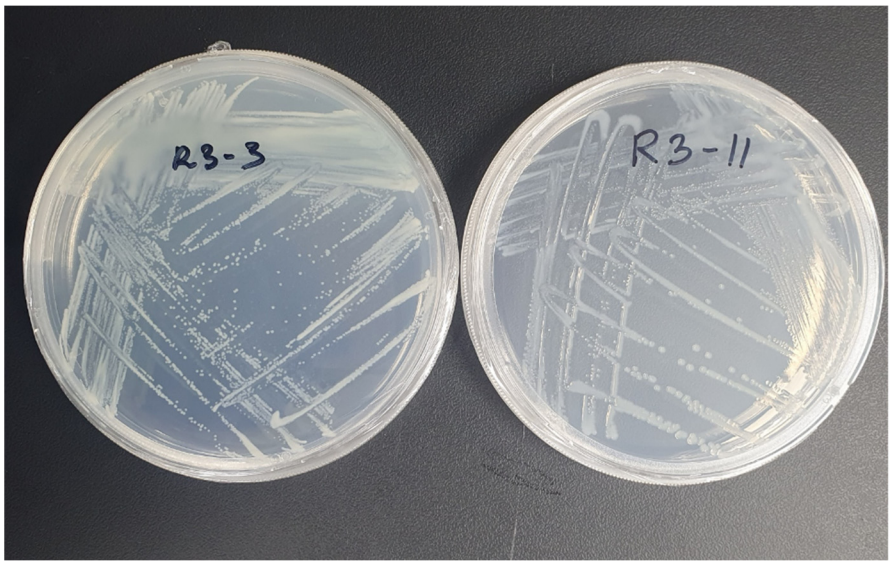

Figure 1. Colonies of R3-3^T and R3-11 were grown on R2A at 28 °C for 72 h.

The predominant quinone in R3-3^T was ubiquinone-8 (Q-8). Polar lipids identified in the analysis included PE (phosphatidylethanolamine), PG (phosphatidylglycerol), DPG (diphosphatidylglycerol), and AL (unidentified aminolipid), along with L (unidentified polar lipid). No glycolipids were detected (Figure S2). The primary fatty acid observed in the strain R3-3^T was $C_{16:0}$ similar to those found in reference strains of members belonging to the genus *Roseateles*, with slight differences in the presence of summed feature 3 and summed feature 8. For detailed results from the fatty acid analyses, refer to Table S2.

Table 1. Distinctive phenotypic traits set apart the newly identified species R3-3^T and R3-11 from its closely related counterparts. Detailed findings from the API ZYM and API 20NE test kits are presented in Table S2. Strain: 1, R3-3^T; 2, R3-11; 3, *Roseateles toxinovorans* (data are from the present study, except fatty acid and G+C content of reference strains, which were retrieved from the literature in parentheses) [6]; 4, *Roseateles koreensis* [8]; 5, *Roseateles albus* [8]; 6, *Roseateles oligotrophus* [7]. +, positive; −, negative; +/−, weak positive or ambiguous; nd, no data.

Characteristics	**1**	**2**	**3**	**4**	**5**	**6**
Isolation source	Fresh soil	Fresh soil	Lake sediment	Fresh water	Fresh water	Fresh water
Temperature range for growth (°C)	10–35	10–35	10–35	15–35	15–28	15–30
Optimal growth temperature (°C)	25–30	25–30	25–30	30	25	25
pH range for growth	5.5–9.5	5.0–9.5	6.0–9.0	6.0–11.0	7.0–12.0	6.0–9.0
pH optimum for growth	7.0–9.0	7.0–9.0	7.0	9.0	9.0	7.0
Highest salt tolerance (%, *w*/*v*)	0.5	0.5	0	0	0	1
Nitrate reduction	−	−	+	+	+	+
Hydrolysis of						
Chitin	−	−	−	−	−	−
Casein	−	−	+	−	+	−
Gelatin	−	−	−	−	+	−
Tween 80	+	+	+	+	+	+
Enzymatic reaction						
Leucine arylamidase	+	+	+	−	+	+
Valine arylamidase	w	w	w	−	−	+
Crystine arylamidase	−	−	w	−	−	+
Alpha chymotrypsin	+	+	+	−	−	+
Acid phosphatase	+	+	w	w	−	+
Naphthol-AS-BI-phosphate	+	+	w	w	w	w
Beta galactosidase	+	+	−	−	−	−
Alpha glucosidase	+	+	−	−	−	−
Beta glucosidase	w	+	−	−	−	+
Alpha monosidase	w	+	−	−	−	−
Assimilation of						
D-Glucose	−	−	−	+	−	−
L-Arabinose	+	+	−	−	−	+
D-Mannitol	+	+	−	−	−	−
D-Maltose	+	+	+	−	−	−
Potassium gluconate	−	−	+	−	−	−
Phenylacetic acid	−	w	+	nd	nd	nd
The major fatty acids (>9%)	$C_{16:0}$, summed feature 3, summed feature 8	$C_{16:0}$, summed feature 3, summed feature 8	$C_{16:0}$, summed feature 4, summed feature 7	$C_{16:0}$, summed feature 3	$C_{16:0}$, summed feature 3	$C_{16:0}$, summed feature 3
DNA G+C content (mol%)	67.5	nd	66.9	61.7	61.8	62.5

3.2. Molecular and Genome Characteristics

The nucleotide sequences of the 16S rRNA genes extracted from strains R3-3^T (1461 nucleotides) and R3-11 (1520 nucleotides) have been archived in the GenBank/EMBL/DDBJ database under the accession numbers PP380088 and PP479825, correspondingly. Analysis of the 16S rRNA gene sequences using the EzBioCloud server for type-material sequences revealed that both strains belong to the family *Comamonadaceae*, specifically within the genus *Roseateles*. They exhibited the highest sequence similarities to *Roseateles puraquae* (97.99%), *Roseateles aquaticus* (97.91%), and *Roseateles saccharophilus* (97.86%). The phylogenetic tree, developed using the 16S rRNA gene sequences, affirmed the positioning of R3-3^T and R3-11 within a separate cluster within the *Roseateles* genus. These strains were observed to exhibit the closest relation to *R. puraquae*, *R. aquaticus*, and *R. saccharophilus*, while showing a distant association with *R. toxinivorans*, *R. koreensis*, *R. oligotrophus*, and *R. albus* (Figures 2, S3 and S4).

Given the limitations of 16S rRNA gene-based phylogeny and similarity in accurately determining phylogenetic relationships, we conducted further analysis of the genome features of R3-3^T. This approach is increasingly recognized as essential, as it provides more reliable criteria for identifying phylogenetically closest relatives of new lineages [29].

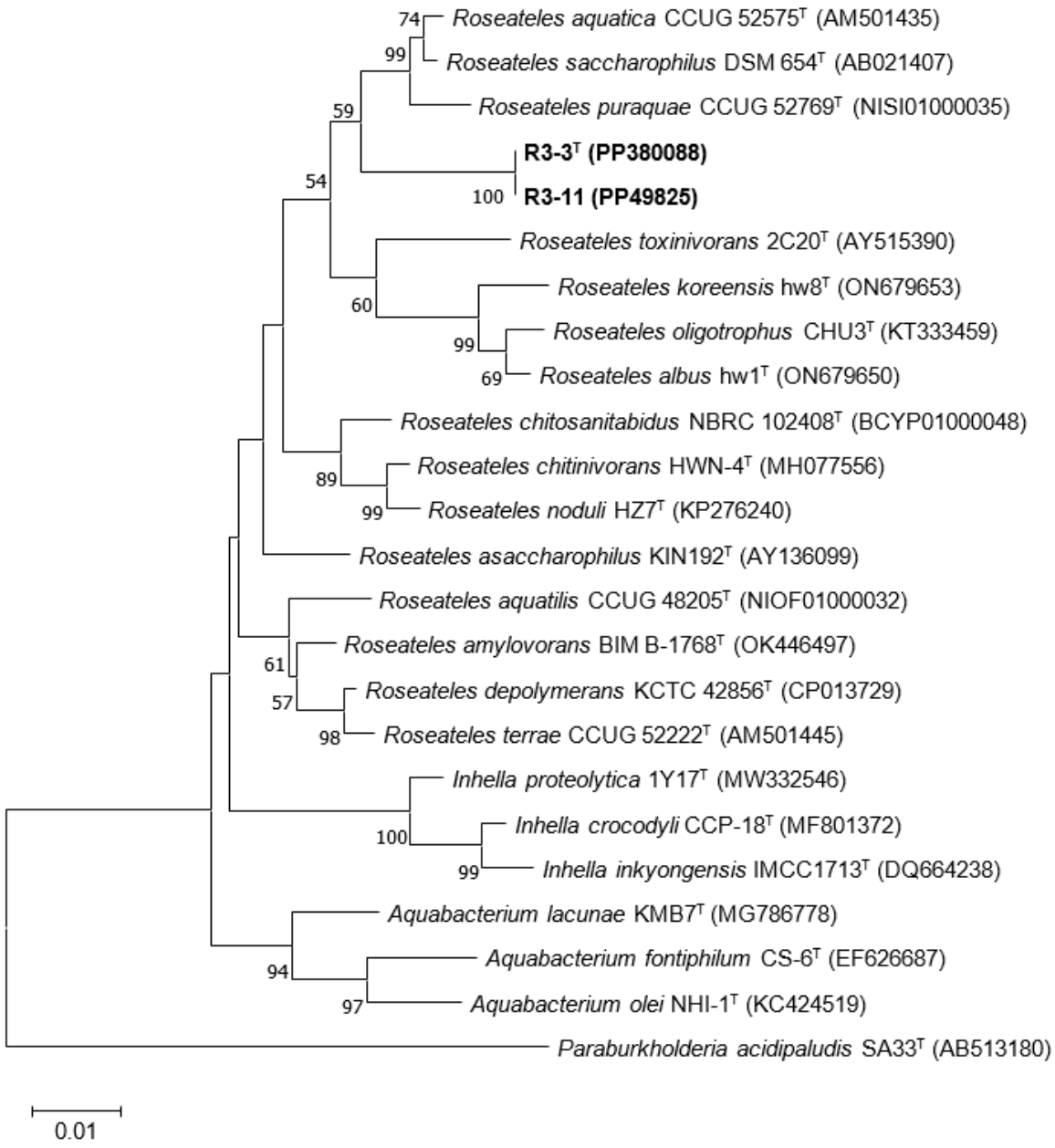

Figure 2. A phylogenetic tree was reconstructed using the minimum evolution method, utilizing 16S rRNA gene sequences from strains R3-3^T, R3-11, and several related strains identified through 16S sequencing. *Paraburkholderia acidipaludis* SAA33^T was included in the analysis as an outgroup. Confidence levels at branch nodes (values > 50%) were indicated based on 1000 replicate bootstrap samplings. GenBank accession numbers were provided in parentheses. The scale bar represents 0.01 substitutions per nucleotide position. The evolutionary analyses were performed using MEGA 11.

Based on core genome-based phylogeny, strain R3-3^T was found to be closely related to *R. puraquae*, *R. aquaticus*, and *R. saccharophilus*, while being distantly grouped with *R. toxinivorans*, *R. koreensis*, *R. oligotrophus*, and *R. albus* (Figure 3). The complete genome of strain R3-3^T comprised 7,061,114 bp, with an N50 of 599,335 kb and a genome coverage of 22.75×. The DNA G+C content was measured at 67.5 mol%. Analysis conducted via RAST unveiled the presence of 6398 coding sequences, 64 RNAs, and 326 subsystems within the genome of strain R3-3^T. Furthermore, 30 polyhydroxybutyrate (PHB) metabolisms

and five secondary metabolisms (one alkaloid biosynthesis from L-lysine and four auxin biosyntheses were found (Figure S5).

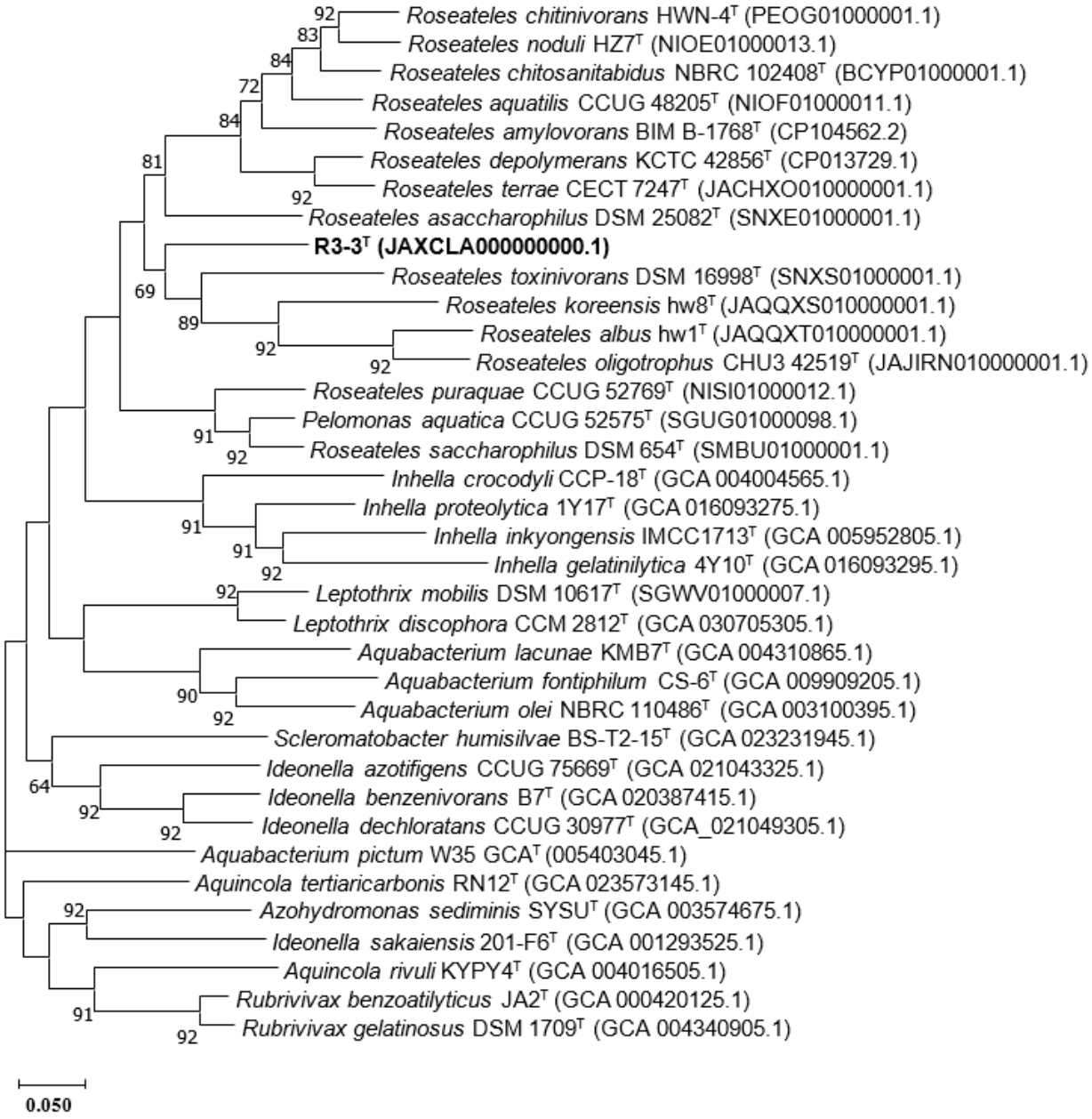

Figure 3. The phylogenetic tree, constructed based on the core genome using UBCG (a concatenated alignment of 92 core genes), delineates the phylogenetic position of strain R3-3^T and its related taxa identified through 16S sequencing. GenBank accession numbers were provided in parentheses. The scale bar represents 0.05 nucleotide substitutions per site.

Concerning the COG category allocation, genes associated with general function prediction constituted 4.1% (239 genes), whereas other identified functions encompassed amino acid transport and metabolism (7.2%—421 genes), inorganic ion transport and metabolism (5.2%—303 genes), and energy production and conversion (5.4%—315 genes). Roughly 30% of the genes fell under unknown functions within the COGs (Figure S6).

A Venn diagram comparison of unique and shared genes among the genomes of R3-3^T, *R. toxinivorans*, *R. koreensis*, *R. oligotrophus*, and *R. albus* indicated that R3-3^T possessed 208 unique genes and shared 2064 common genes with all four strains (Figure 4).

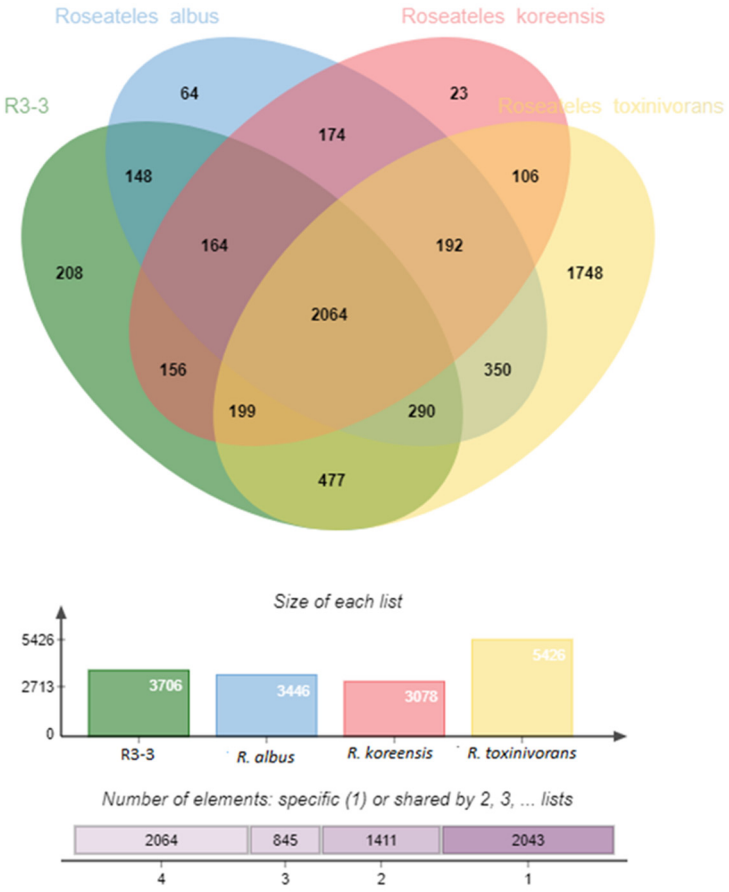

Figure 4. Venn diagram of whole-genome orthologous genes in R3-3^T and *R. toxinivorans*, *R. koreensis*, and *R. albus*. The numbers in the diagram indicate overlapped conserved genes or non-overlapped unique genes in each species. The numbers below the strain names identify the total number of protein-coding genes within each genome.

Comparing pairwise ANI and dDDH values between strain R3-3^T and other type strains within the *Roseateles* genus revealed ANI values spanning from 75.14% to 78.30%, and dDDH values ranging from 20.70% to 22.70% (Table S3). These values were below the suggested threshold values of ANI 95–96% [21] and dDDH 70% [30] for species delineation, suggesting that the isolated strain constitutes a separate species within the *Roseateles* genus.

4. Conclusions

Phylogenetic analysis categorized the R3-3^T isolate within the *Roseateles* genus. Additionally, upon considering additional phenotypic traits, strain R3-3^T exhibited clear distinctions from existing strains, as outlined in Table 1. Moreover, pairwise ANI values between strain R3-3^T and other type strains within the *Roseateles* genus were $\leq$78.30%, significantly falling below the commonly acknowledged species threshold of 95%. Similarly, the findings of digital DNA–DNA hybridization (dDDH) between strain R3-3^T and other type strains within the *Roseateles* genus ranged from 20.70 to 22.0%, well beneath the recognized species threshold of 70%, indicating considerable genomic disparities among them.

To sum up, strain R3-3^T exhibited numerous distinctive traits that differentiate it from its closest phylogenetic neighbors and other closely related members of the *Roseateles* genus. According to the polyphasic data provided above, strain R3-3^T could be proposed as a new species within the *Roseateles* genus, named *Roseateles agri* sp. nov.

Description of Roseateles agri sp. nov.

Roseateles agri (a'gri. L. gen. masc. n. *agri*, of a field).

Cells of R3-3^T exhibit characteristics such as being Gram-negative, rod-shaped, facultatively anaerobic, with dimensions approximately ranging from 1.4 to 3.5 μm in length and 0.5 to 1.0 μm in width. Colonies cultured on R2A medium appear white, circular, convex, glistening, with regular margins and smooth surfaces, typically ranging in size from 0.5 to 2.5 mm after 3 days of incubation. Optimal growth occurs within the temperature range of 10 °C–35 °C (with the preferred range being 25 °C–30 °C) and pH range of 5.0 to 9.5 (optimal pH being 7.0–9.0), with the highest tolerance to salt at 0.5% NaCl (optimal at 0%). Catalase and oxidase activities test positive. Growth is observed exclusively on R2A medium, while no growth occurs on TSA, LB, NA, Mueller–Hinton agar (MB Cell), and MacConkey agar. Positive reactions are observed for the hydrolysis of Tween 80 and DNA, while negative results are obtained for starch, casein, chitin, and gelatin hydrolysis.

According to the API 20NE system, β-Galactosidase and assimilation of L-Arabinose, D-mannitol, and D-Maltose are positive. Urea hydrolysis shows weak positivity, while nitrate is not reduced to nitrite, and indole production, fermentation of D-glucose, esculin ferric citrate, and assimilation of D-Glucose, D-mannose, N-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, trisodium citrate, and phenylacetic acid are negative. In the API ZYM system, positive enzyme activities include alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β galactosidase, α-glucosidase, β-glucosidase, and α-monosidase. Weak positivity is observed for valine arylamidase, while other enzyme activities such as lipase C14, cystine arylamidase, trypsin, α-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, and α-fucosidase test negative.

The designated type strain is R3-3^T (= KACC 23678^T = NBRC 116681^T), which was isolated from agricultural soil in Cheongggye-dong, Uiwang-si, Gyeonggi-do, Republic of Korea. The genome measures 7,061,114 bp in size, with a G + C content of 67.5 mol%. The GenBank accession numbers for sequences pertaining to strain *Roseateles agri* R3-3^T are JAXCLA000000000 for the genome and PP380088 for the 16S rRNA gene nucleotide sequence.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/d16050279/s1, Figure S1: Colonies of R3-3^T and R3-11 were grown on R2A at 28 °C for 72 h; Figure S2: Transmission electron microscopy of strain R3-3^T grown on R2A medium plates for 2 days at 28 °C; Figure S3: Polar lipid profile of strain R3-3^T; Figure S4: The phylogenetic tree was reconstructed with the neighbor-joining method based on 16S rRNA gene sequences of strains R3-3^T, R3-11, and some related strains based on 16S sequencing; Figure S5: Phylogenetic tree reconstructed with the maximum likelihood method based on 16S rRNA gene sequences of strains R3-3^T, R3-11, and some related strains based on 16S sequencing; Figure S6: COG functional classification of proteins in strains R3-3^T genome; Table S1: Results from API ZYM, API 20NE test; Table S2: Detailed cellular fatty acid profiles (% of totals) of strain 1. R3-3^T AND 2. R3-11; Table S3: The average nucleotide identity and digital DNA–DNA hybridization value values between strains R3-3^T and related taxa.

Author Contributions: Y.Y. formulated, planned, and executed all experimental procedures. Y.Y. and J.K. analyzed the data, participated in discussions, revised the manuscript, and endorsed its final version. J.K. oversaw and managed the study. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

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