

**Gottfriedia endophyticus** sp. nov., a novel indole-acetic acid producing bacterium isolated from the roots of rice plant

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**Abstract** A Gram-stain-positive, aerobic, motile and rod-shaped bacterium, designated RG28\(^\text{T}\), was isolated from the roots of rice plant collected from paddy fields in Ilsan, South Korea. Cells of the strain were oxidase-negative but catalase-positive. Strain RG28\(^\text{T}\) was found to grow at 10–50 °C (optimum, 25–30 °C), pH 5.0–10.0 (optimum, pH 7.0) and in 1.0–5.0% (w/v) NaCl (optimum, 0%). The cell-wall peptidoglycan contained meso-diaminopimelic acid and the predominant menaquinones were MK-7 and MK-6. The predominant cellular fatty acids were \(\text{C}_{16:0}\), iso-\(\text{C}_{15:0}\) and anteiso-\(\text{C}_{15:0}\). The major polar lipids included phosphatidylethanolamine, diphasphatidylglycerol, phosphatidylglycerol, four unidentified aminophosphoglycolipids, four unidentified aminophospholipids, two unidentified glycolipids, one unidentified aminoglycolipid and four unidentified lipids. The genomic DNA G+C content was 33.5 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that the strain was closely related to *Gottfriedia acidiceleris* CBD 119\(^\text{T}\) (98.6%), *Gottfriedia solisilvae* LMG 18422\(^\text{T}\) (98.5%) and *Gottfriedia luciferensis* LMG 18422\(^\text{T}\) (98.4%). The average nucleotide identity (ANI) and in silico DNA–DNA hybridization (isDDH) values between strain RG28\(^\text{T}\) and type strains of *Gottfriedia* species were lower than the cut-offs (≥ 95–96% for ANI and ≥ 70% for isDDH) required to define a bacterial species. Meanwhile, the strain has the ability to produce indole-acetic acid (40.5 µg/mL). Phylogenetic, physiological and chemotaxonomic data suggested that strain RG28\(^\text{T}\) represented a novel species of the genus *Gottfriedia*, for which the name *Gottfriedia endophyticus* sp. nov. is proposed, with the type strain RG28\(^\text{T}\) (= KCTC 43327\(^\text{T}\) = TBRC 15151\(^\text{T}\)).

**Keywords** *Gottfriedia* · *Gottfriedia endophyticus* · Endospore · Indole-acetic acid · Tryptophan · Biofertilizer

**Abbreviations**

| Abbreviation | Description |
|--------------|-------------|
| ANI | Average nucleotide identity |
| NJ | Neighbour joining |
| ML | Maximum-likelihood |
| ME | Minimum-evolution |
| MP | Maximum-parsimony |
| KEMB | Korean environmental microorganisms bank |

**Repositories** The draft genome and 16S rRNA gene sequences of strain RG28\(^\text{T}\) have been deposited in GenBank/EMBL/DDBJ under accession numbers JAGIYQ00000000 and MW386408 respectively.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s10482-022-01748-2.
Introduction

Members of genus Gottfriedia were transferred from the polyphyletic genus Bacillus, whose complicated interspecies taxonomy arose as a result of vague criteria used to assign novel bacteria into the genus (Jiang et al. 2021). As a result, Bacillus has been restricted only include species closely related to Bacillus subtilis and Bacillus cereus, and other species were transferred into other novel genera. The name Gottfriedia was chosen to celebrate the German scientist and naturalist Christian Gottfried Ehrenberg, who initially proposed the name Bacillus for rod-shaped and to recognize his contributions to the studies of microscopic organisms (Gupta et al. 2020). Members of the genus Gottfriedia form a monophyletic clade in phylogenetic trees based on concatenated sequences for several large datasets of proteins which differentiated this genus from other Bacillaceae genera. The type species for this genus is Gottfriedia luciferensis (Logan et al. 2002). As of April 2021, there are a total of only three species with validly published names (https://lpsn.dsmz.de/genus/gottfriedia). Species of Gottfriedia have been isolated from soils and forensic specimens so far (Logan et al. 2002; Pan et al. 2017; Peak et al. 2007). Gottfriedia is a genus of Gram-stain positive, rod-shaped, aerobic or facultatively anaerobic, cream to grey color and endospore-forming bacteria with low G+C content ranges from 32 to 66 mol% (Pan et al. 2017). Major polar lipids of this genus are phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. Menaquinone-7 (MK-7) are the major respiratory quinone (Logan et al. 2002; Pan et al. 2017). Here, we present the isolation and description of a novel, indole-3-acetic acid (IAA) producing bacterium belonging to the genus Gottfriedia recovered from the roots of rice plants. In continuation to our previous work 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. Since the strain RG28T was isolated from the roots, IAA production was investigated. The plant hormone IAA plays a role in the communication between host plant and microbes, including plant-associated microorganisms and endophytes but as well as plant pathogens (Rai et al. 2005; Vandeputte et al. 2005). In this study we also compared the amount of IAA produced by strain RG28T and other three close strains in the genus Gottfriedia.

Materials and methods

Isolation and ecology

Samples from paddy field were collected near Dongguk University, Ilsan, South Korea, (GPS coordinates of the sample collection site: 37° 40’ 26.4″ N 126° 48’ 20.88″ E) for bacterial isolation as part of an ongoing study on the microbial diversity in our lab (Chhetri et al. 2019, 2020, 2021a, b). Roots were gently washed with water to remove adhered soil and prepared for screening of novel isolates as described previously (Chhetri et al. 2021b). The root samples were surface-sterilized and after being dried in the hood and the surface sterilized samples were ground into powder by mortar and pestle. The macerated samples were serially diluted using 0.85% NaCl. Isolation was achieved using R2A agar (MB-RR1129) containing (g/L) 0.5 g yeast extract, 0.5 g proteose peptone No. 3, 0.5 g casamino acid, 0.5 g dextrose, 0.5 g soluble starch, 0.3 g dipotassium phosphate, 0.05 g magnesium sulfate heptahydrate, 0.3 g sodium pyruvate and 15 g agar at 28°C for 1 week. A single colony chosen on the plates was purified by transferring to new R2A plates. From the purified bacterial colonies, a novel strain of the genus was identified to be a member of Gottfriedia and was designated as RG28T. Purified colonies were cultured routinely on R2A at 30 °C and preserved as a suspension in R2A broth with glycerol (25%, v/v) at −80 °C.

16S rRNA gene sequence similarities and phylogenetic analysis

The genomic DNA was extracted and PCR amplification and sequencing of 16S rRNA gene were performed as described previously (Kim et al. 2020a, b). The 16S rRNA gene of the isolate was directly amplified by colony-PCR using the universal bacterial primers 27F, 518F, 805R and 1492R; PCR products were commercially sequenced (Solgent, Korea). EzBioCloud’s Identify service (www.ezbiocloud.net/
identify; Kim et al. 2012) was used to identify strain RG28T, and the 16S rRNA gene sequences of closely related type strains were retrieved. These sequences were aligned by using the CLUSTAL_X program (Thompson et al. 1997). Phylogenetic trees were reconstructed using the neighbour-joining (NJ), maximum-likelihood (ML), minimum-evolution (ME) and maximum-parsimony (MP) methods in the software package mega version 7.0 (Saitou et al. 1987; Felsentein et al. 1981; Rzhetsky et al. 1992; Fitch et al. 1971; Kumar et al. 2016). The robustness of the topologies for the phylogenetic trees was evaluated by bootstrap analysis of 1000 replications (Felsenstein et al. 1985).

Genome sequencing, assembly and annotation

Genomic DNA sequencing was performed at Macrogen on an Illumina Hiseq4000 system. A DNA library was prepared using the TruSeq Nano DNA kit. Raw reads filtered by FastQC and were assembled using SOAPdenovo v. 3.10.1 de novo assembler. After assembling the draft genome, the locations of protein genes were predicted and their functions were annotated. The average nucleotide identity (ANI) values between strain RG28T and its phylogenetic neighbours were calculated using an ANI calculator (www.ezbiocloud.net/tools/ani) (Yoon et al. 2017). The estimated digital DNA–DNA hybridization (dDDH) values were calculated using the Genome-to-Genome Distance Calculator (GGDC2.1, http://ggdc.dsmz.de/distcalc2.php) (Meier-Kolthoff et al. 2013). The DNA G+C content of strain RG28T was determined according to the genomic DNA sequences. Gene prediction and annotation were performed using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (Tatusova et al. 2016). Comparisons of orthologous gene clusters among strain RG28T and other close strains were performed by using OrthoVenn2 (https://orthovenn2.bioinfotoolkits.net/home) (Xu et al. 2019). In order to strengthen the phylogenetic status and better characterize the relationships between the novel isolate and its other closely related species, phylogenomic trees were constructed based on the basis of an up-to-date bacterial 92 core gene set (UBCG) (Na et al. 2018). The secondary metabolic gene clusters were analysed with antiSMASH (version 2.0.2) (Blin et al. 2019).

Phenotypic features

Cells of RG28T grown on R2A at 30 °C for three days were used for physiological and biochemical tests. Colony morphology was observed on R2A agar plates after incubation at 30 °C. Cell morphology and flagellum was examined by growing the cells for three days at 30 °C using transmission electron microscopy (TEM) (LIBRA 120, Carl Zeiss, Germany). For the latter assessments, the cells were negatively stained with 1% (w/v) phosphotungstic acid and, after air-drying, the grids were examined for cell morphology. Anaerobic growth on R2A medium was evaluated using the GasPack anaerobic system (BBL, Cockeysville, MD, USA). Formation of endospores was assessed with the malachite green stain after cell growth on R2A for 3 days. Oxygen absorber stripes (MITSUBISHI GAS CHEMICAL company) were used to remove oxygen. Growth at various temperatures (4–55 °C) was measured on R2A agar for ten days. 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, Reasoner’s 2agar (R2A; MB-R1129), marine agar (MA; MB-M1505), nutrient agar (NA; Difco-213000), tryptic soy agar (TSA; MB-T1053) and Luria–bertani agar (LB; MB-L4488) 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/Na2HPO4 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. 2020a, b). The activities of catalase and oxidase and hydrolysis of casein, chitin, carboxymethyl-cellulose, starch and Tweens 20, 40, 60 and 80 were determined as described previously (Smibert et al. 1994). In addition, the strain RG28T and reference strains were characterized biochemically using API 20NE and API ZYM strips (bioMérieux), according to the manufacturer’s instructions. Acid production from carbon sources was tested using the API 50CH (bio-Mérieux) system.

Chemotaxonomic analysis

The cellular fatty acid profiles of strains RG28T and its close strains were determined using cells from the third
quadrants grown on R2A medium at 30 °C for 48 h. The cells were saponified, methylated and extracted using the standard MIDI (Sherlock Microbial Identification System, version 6.0B) protocol as described previously (Collins et al. 1981). Isoprenoid quinines were extracted with methanol/water and petroleum ether at 60–80 °C, evaporated under a vacuum. The sample was re-extracted with acetone and analysed by HPLC as described previously (Kuykendall et al. 1988). For peptidoglycan analysis, cells of strain RG28T were grown in R2A broth on a rotator shaker for 4 days at 30 °C and performed as described previously (Kim et al. 2019). Polar lipids were extracted and separated using two-dimensional TLC according to the method described previously (Minnikin et al. 1984). The solvent systems of the first and the second dimension were chloroform–methanol–water (64:27:5, by vol.) and chloroform–acetic acid–methanolwater (80:18:12:5, by vol.), respectively. To identify the specific moieties of lipids the following spraying methods were applied: 0.5% a-naphthol in methanol and water (1:1, v/v) followed by spraying with 95% sulfuric acid for glycolipids; 0.25% ninhydrin in acetone for amino lipids; molybdenum blue reagent (Sigma) for phospholipids; and 5% molybdatophosphoric acid hydrate (Merck) in ethanol for total lipids (Komagata et al. 1987).

Indole acetic acid (IAA) production and quantification

Strain RG28T and all three reference strains were grown in R2A 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. Reserved supernatant was spectrophotometrically assessed at 530 nm. Indole production was indicated by color change into orange to pink. Result was compared with and without tryptophan (not shown).

Results and discussion

16S rRNA gene sequence similarities and phylogenetic analysis

An almost full-length sequence of the 16S rRNA gene (1484 bp) was determined for strain RG28T, which has been stored in the GenBank databases under the accession number MW386408. The full length of the 16S rRNA gene of strain RG28T showed the highest sequence similarity to G. acidiceleris CBD 119T (98.6%), G. solisilvae LMG 18422T (98.5%) and G. luciferensis LMG 18422T (98.4%). Sequence similarities to all other species of the genus Bacillus, Cytopbacillus, Neobacillus, Sutcliffiella and Metabacillus were below 95.2%. The NJ phylogenetic analysis revealed that strain RG28T formed a separate cluster with G. acidiceleris CBD 119T, G. solisilvae LMG 18422T and G. luciferensis LMG 18422T with high topology which was also supported by the trees reconstructed using the ML, ME and MP methods (Fig. 1, Fig S1, Fig S2 and Fig S3). The position of strain RG28T did not vary with the tree reconstruction method used. Reference strains Gottfriedia acidiceleris KEMB 1602-188T, G. luciferensis KEMB 7305-013T and G. solisilvae DSM 100485T obtained from Korean Environmental Microorganisms Bank (KEMB) and Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) were used for subsequent comparison.

Genome sequencing, assembly and annotation

The draft genome of strain RG28T was 4,081,222 bp long with a G+C content of 33.5 mol% and consisted of 34 contigs. The N50 length was 362,561 bp. A total of 3940 genes were predicted with 3802 protein-coding genes and 15 RNAs (seven 5S rRNAs, four 16S rRNAs, four 23S rRNAs), 73 tRNAs and three ncRNAs. The ANI value between strain RG28T and G. acidiceleris KEMB 1602-188T, G. solisilvae DSM 100485T and G. luciferensis KEMB 7305-013T were 74.7, 74.8 and 74.9%, respectively, clearly below the recommended cut-off value of 95–96% for species identification (Goris et al. 2007). The estimated dDDH value between strain RG28T and G. acidiceleris KEMB 1602-188T, G. solisilvae DSM 100485T and G. luciferensis KEMB 7305-013T were 21.6, 21.8 and 21.9%, respectively, which was well below
These results indicated that strain RG28\textsuperscript{T} represents a novel species of the genus *Gottfriedia*. The overall comparison analysis result is displayed as Venn diagram in Fig. 2. A total of 2299 orthologous genes were shared among all the compared species, of which 79 were shared only between strain RG28\textsuperscript{T} and *G. acidiceleris* KEMB 1602-188\textsuperscript{T}, 77 between strain RG28\textsuperscript{T} and *G. solisilvae* DSM 100485\textsuperscript{T} and 71 between strain RG28\textsuperscript{T} and *G. luciferensis* KEMB 7305-013\textsuperscript{T}. According to the genome-based phylogeny, strain RG28\textsuperscript{T} formed a clade with *G. acidiceleris* KEMB 1602-188\textsuperscript{T}, *G. solisilvae* DSM 100485\textsuperscript{T} and *G. luciferensis* KEMB 7305-013\textsuperscript{T} in the genus *Gottfriedia* with bootstrap support of 92\%, confirming the topology determined by 16S rRNA gene sequencing. Furthermore, four *Gottfriedia* species formed a monophyletic cluster and were clearly separated from other species of the genus *Bacillus*, *Neobacillus* and *Cytobacillus* (Fig S4). AntiSMASH analysis results showed four gene clusters within the genome of strain RG28\textsuperscript{T}, namely two gene cluster for terpene, one gene cluster for thiopeptides and one gene cluster for linear azol (in) e-containing peptide (LAP). When the results of secondary metabolic gene clusters were compared between strain RG28\textsuperscript{T} and its closest relatives, the gene cluster for thiopeptide was only found in strain RG28\textsuperscript{T} which distinguish the novel isolates from its close relatives. In addition, large number of genes involve in sporulation, spore formation were also detected in the genome of strain RG28\textsuperscript{T} (Table S1), which is consistent with our results showing the production of endospores. The genome of strain RG28\textsuperscript{T} contained four genes related to tryptophan biosynthesis: tryptophan synthase subunit beta (*trpB*) (JAGIYQ010000010), tryptophan–tRNA ligase (*trpS*) (JAGIYQ010000003), tryptophan 2,3-dioxygenase (JAGIYQ010000006) and anthranilate synthase component I (JAGIYQ0100000021) which indicates that strain RG28\textsuperscript{T} could contribute to the plant growth-promoting activity in rice plants. When we compared the plant growth promoting rhizobacteria (PGPR) genes of strain RG28\textsuperscript{T} with its close strains, interestingly the reference strains had more genes related to tryptophan biosynthesis. In addition, the reference strains had genes for siderophore and indole biosynthesis which were not found in the genome of strain RG28\textsuperscript{T} (Table 1). These genomic features indicate that strain RG28\textsuperscript{T} and its reference strains could be a PGPR candidate.

**Phenotypic features**

Strain RG28\textsuperscript{T} was Gram-stain-positive, catalase-positive and oxidase-negative. Colonies grown on R2A plates were circular, white, smooth and 1–3 mm in
diameter after three days of culture. Cells of the isolate were motile with peritrichous flagella (Fig. S5). Cells were able to grow at 10–50 °C and pH 5.0–10.0, with optimal growth at 25–30 °C and pH 7.0. The growth occurred in 0–5% NaCl (w/v) with optimum 0%. The strain showed no anaerobic growth on R2A plates. Endospores were produced at the termini in non-swollen sporangia (Fig. S6). Strain RG28T grew well on R2A, NA, TSA and LB but grew only moderately on MA. Strain RG28T was tolerant up to 5% NaCl, while G. solisilvae DSM 100485T tolerated to 4%, G. acidicerleris CBD 119T to 2% and G. luciferensis DSM 18422T to 1% of NaCl in culture media. Cell morphology, colony colour, presence of flagella, oxidase activity, temperature and pH range for growth, differentiates the strain RG28T from its close strains. The detailed physiological and biochemical characteristics of strain RG28T are summarized in the species description and characteristics that differentiated strain RG28T from its closest related type strains are listed in Table 2.

Chemotaxonomic analysis

The whole-cell fatty acid profile of strain RG28T contained large amounts of C16:0 (25.5%), iso-C15:0 (26.4%) and anteiso-C15:0 (33.4%). A comparison of strain RG28T with closely related members of the genus Gottfriedia is presented in Table S2. Strain RG28T had a similar fatty acid profile to G. acidicerleris KEMB 1602-198T, G. solisilvae DSM 100485T and G. luciferensis KEMB 7305-013T, but the absence of C18:0 and iso-C13:0 in strain RG28T distinguishes it from its close relatives. The peptidoglycan of strain RG28T contained meso-DAP as the diagnostic diamino acid. The predominant menaquinone was MK-7 (57%), followed by MK-6 (43%). The quinone and peptidoglycan diamino acid of strain RG28T as found in all known members of the genus Gottfriedia. However, strain RG28T could be distinguished from the reference strains with the proportions of MK-7 and MK-6. In addition, G. acidicerleris CBD 119T has MK-8, which is not found in strain RG28T and other two reference strains, which clearly differentiates them from each other (Table 2). The polar lipid profile of strain RG28T consist of diphosphatidylglycerol (DPG), phosphatidy ethanolamine (PE), phosphatidylglycerol (PG), four unidentified aminophosphoglycolipids (APGL1-4), four unidentified aminophospholipids (APL1-4), two unidentified glycolipids (GL1-2), one unidentified aminoglycolipid (AGL) and four unidentified lipids (L1-4) (Fig. S7). The major polar lipid profile was same with other three reference strains however the presence of other minor lipids distinguish the strain RG28T from other closely related strains (Pan et al. 2017).

Indole acetic acid (IAA) production and quantification

Change in color showed that strain RG28T and its reference strains G. acidicerleris KEMB 1602-198T, G. solisilvae DSM 100485T and G. luciferensis KEMB 7305-013T showed the ability to synthesize IAA only in the presence of the precursor L-tryptophan and could produce 40.5, 55.2, 56.8 and 50.5 µg/ml IAA, respectively (Fig. 3). To the best of our knowledge,
no reports are available for plant growth activities of genus *Gottfriedia* so far, in this study we found that all strain of genus *Gottfriedia* including novel strain RG28<sup>T</sup> were able to produce IAA in sufficient amounts. Genome annotation also revealed the number of genes associated with tryptophan biosynthesis which is consistent with our results which suggest a potential use of *Gottfriedia* species as biofertilizer.

### Taxonomic conclusion

Phylogenetic and phylogenomic analysis indicated that the strain RG28<sup>T</sup> formed a different cluster with the three members of genus *Gottfriedia* with high topology. Based on the above polyphasic taxonomic analysis, strain RG28<sup>T</sup> was confirmed as a novel species in the genus *Gottfriedia*. Therefore, the name *Gottfriedia endophyticus* sp. nov. is proposed.

### Description of *Gottfriedia endophyticus* sp. nov.

*Gottfriedia endophyticus* (en.do.phy′ti.cus. Gr. pref. Endo within; G. n. phyton plant; L. masc. suff. –icus adjectival suffix used with the sense of belonging to; N.L. masc. adj. *endophyticus* within plant, endophytic, pertaining to the original isolation from plant tissues).

Cells are short to long-rods, Gram-positive, aerobic, capable of forming ellipsoidal endospores and motile by peritrichous flagella. Colonies on R2A agar are moist, flat, white and undulate in margins after three days of incubation at 30 °C. Growth occurs at 10–50 °C (optimum, 25–30 °C) and pH of 5.0–10.0 (optimum, pH 7.0) and tolerates up to 5.0% NaCl. NaCl is not required for growth. Cells were positive for hydrolysis of casein, starch, CM-cellulose and Tween 80 but negative for chitin hydrolysis. Cells are positive for catalase and negative for oxidase activity. According to the API ZYM system, cells were positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-glucuronidase, β-glucosidase. In the API 20NE system, all phenotypic characteristics are negative except for indole production, arginine dihydrolase, esculin hydrolysis and β-galactosidase. In the API 50CH, following compounds are utilized as sole source of carbon and energy: D-ribose, D-glucose, N-acetylglucosamine, esculin, slicine, D-maltose and 5-keto-gluconate. Menaquinone-7 (MK-7) and meso-diaminopimelic

| Prokaryotes | Proteins | 1 | 2 | 3 | 4 |
|-------------|----------|---|---|---|---|
| Tryptophan | Tryptophan synthase subunit beta trpB | J5Y03_RS14090 | B6K90_RS09040 | CAB05_RS14505 | B7R67_RS11880 |
|           | Tryptophan-ρRNA ligase trpS          | J5Y03_RS05225 | B6K90_RS07255 | CAB05_RS1475 | B7R67_RS00505 |
|           | Tryptophan 2,3-dioxygenase            | J5Y03_RS10820 | B6K90_RS19860 | CAB05_RS13600 | B7R67_RS21350 |
|           | Tryptophan synthase subunit alpha trpA | – | B6K90_RS09035 | CAB05_RS14500 | B7R67_RS11885 |
|           | N-methyl-L-tryptophan oxidase solA    | – | B6K90_RS1475 | – | – |
|           | Tryptophan-rich sensory protein       | – | B6K90_RS15130 | CAB05_RS10035 | B7R67_RS00150 |
|           | Anthranilate phosphoribosyltransferase trpD | – | B6K90_RS09055 | CAB05_RS14520 | B7R67_RS11865 |
|           | Anthranilate synthase component I trpE | J5Y03_RS18995 | B6K90_RS09065 | CAB05_RS14530 | B7R67_RS02035 |
|           | Aminodeoxychorismate/anthranilate synthase component II trpG | – | B6K90_RS09060 | CAB05_RS14525 | – |
| Indole    | Indole-3-glycerol phosphate synthase trpC | – | B6K90_RS09050 | CAB05_RS14515 | B7R67_RS11870 |
| Siderophore | Iron-siderophore ABC transporter substrate-binding protein | – | B6K90_RS18340 | CAB05_RS13705 | B7R67_RS14995 |

### Table 1 Presence of genes associated with IAA and siderophore in genomes of strain RG28<sup>T</sup> and its reference strains

### Strain: 1. RG28<sup>T</sup>; 2, *G. acidiceleris* KEMB 1602-188<sup>T</sup>; 3, *G. solisilvae* DSM 100485<sup>T</sup>; 4, *G. luciferensis* KEMB 7305-013<sup>T</sup>
### Table 2  Differential characteristics of strain RG28\textsuperscript{T} and closely related type strains of the genus *Gottfriedia*

| Characteristics               | 1 | 2 | 3 | 4 |
|------------------------------|---|---|---|---|
| Isolation source             | Roots | Forensic specimen | Forest soil | Volcanic soil |
| Cell morphology              | Rods occurring singly or in pairs | Rods occurring in pairs or short chains with branches* | Rods occurring singly or in pairs | Rods occurring singly or in pairs |
| Colony colour                | Cream | Cream to pearly grey | Cream | Creamy-grey |
| Swollen sporangia            | + | + | + | – |
| Motility                     | + | – | + | + |
| Catalase/oxidase             | ± | +/+ | ± | ± |
| Temperature range for growth (°C) | 10–50 | 15–45 | 15–40 | 15–45 |
| pH range for growth          | 5.0–10.0 | 6.0–8.0 | 6.0–8.0 | 5.0–9.0 |
| NaCl (%) tolerance           | 0–5 | 0–2 | 0–4 | 0–1 |
| *Hydrolysis of*              |   |   |   |   |
| Gelatin                      | – | + | – | + |
| Urea                         | – | + | – | – |
| Casein                       | + | + | – | + |
| Starch                       | + | – | + | + |
| CM-cellulose                 | + | – | – | + |
| Tween 80                     | + | – | + | + |
| *Enzyme activity*            |   |   |   |   |
| Esterase Lipase (C8)         | + | – | – | + |
| Lipase (C14)                 | – | + | + | – |
| Valine arylamidase           | – | + | + | – |
| Cystine arylamidase          | – | + | + | + |
| α-Chymotrypsin               | + | – | + | + |
| α-Galactosidase              | – | – | – | + |
| N-acetyl-β-glucosaminidase   | – | + | – | – |
| *API 20NE:*                  |   |   |   |   |
| Glucose fermentation         | – | + | + | – |
| β-Galactosidase              | + | – | + | + |
| L-Arabinose                  | – | + | + | – |
| *Acid production from (API 50 CH)* |   |   |   |   |
| D-Arabinose                  | – | + | + | + |
| D-Glucose                    | + | – | – | + |
| D-Trehalose                  | – | + | + | + |
| D-Turanose                   | + | + | – | – |
| D-Xylose                     | – | + | – | – |
| L-Arabinose                  | – | + | + | + |
| L-Rhamnose                   | – | + | + | + |
| L-Xylose                     | – | + | – | – |
| Inositol                     | + | – | – | + |
| Methyl-D-mannopyranoside     | – | + | – | – |
| Glycogen                     | – | + | + | – |
| Xylitol                      | – | + | + | + |
Table 2 (continued)

| Characteristics | 1 | 2 | 3 | 4 |
|-----------------|---|---|---|---|
| Menaquinone types | MK-7 (57%), MK-6 (43%) | MK-7 (53%), MK-6 (31%), MK-8 (16%) | MK-7 (54%), MK-6 (46%) | MK-6 (64%), MK-7 (36%) |
| DNA G+C content (%) | 33.5 | 32.8 | 33 | 32.8 |

Strain: 1. RG28T; 2. G. acidiceleris KEMB 1602-188T; 3. G. solisilvae DSM 100485T; 4. G. luciferensis KEMB 7305-013T. Data were taken from this study unless otherwise indicated. +, Positive; –, negative. Data for DNA G+C content (%) were covered from NCBI, whole genome annotation.

*Data taken from Peak et al. 2007*

glycolipids, one unidentified aminoglycolipid and four unidentified lipids.

The type strain is RG28T (= KCTC 43327T = TBRC 15151T) which was isolated from the roots of rice plant collected from Ilsan region, South Korea. The DNA G+C content of the type strain is 33.5%. The GenBank accession number for the 16S rRNA gene sequence is MW386408, and the genome accession number is JAGIYQ00000000.

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Declarations

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

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

References

Blin K, Shaw S, Steinke K et al (2019) antiSMASH 5.0: updates to the secondary metabolite genome mining pipeline. Nucleic Acids Res 47:W81–W87

Chhetri G, Kim J, Kim H, Kim IH, Seo T (2019) Pontibacter oryzae sp. nov., a carotenoid-producing species isolated from a rice paddy field. Antonie van Leeuwenhoek 112:1705–1713

Chhetri G, Kim J, Kim I, Kim H, Seo T (2020) Adhaeribacter rhizoryzae sp. nov., a fibrillar matrix producing bacterium isolated from the rhizosphere of rice plant. Int J Syst Evol Microbiol 70:5382–5388

Chhetri G, Kim J, Kim I, Kang M et al (2021a) Flavobacterium baculatum sp. nov., a carotenoid and flexirubin-type
pigment producing species isolated from flooded paddy field. Int J Syst Evol Microbiol 71:004736

Chhetri G, Kim J, Kim I, Kang M, Seo T (2021b) Limnohabitans radicola sp. nov., a slow-growing bacterium isolated from rhizosphere of rice plant and emended description of the genus Limnohabitans. Int J Syst Evol Microbiol 71:4957

Chun J, Oren A, Ventosa A, Christensen H, Arahal DR et al (2018) Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int J Syst Evol Microbiol 68:461–466

Collins MD, Jones D (1981) Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implications. Microbiol Rev 45:316–354

Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17:368–376

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Fitch WM (1971) Toward defining the course of evolution: minimum change for a specific tree topology. Syst Zool 20:406–416

Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P et al (2007) DNA–DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol 57:81–91

Gupta RS, Patel S, Saini N, Chen S (2020) Robust demarcation of 17 distinct Bacillus species clades, proposed as novel Bacillaceae genera, by phylogenomics and comparative genomic analyses: description of Roberimururraya kyonggiensis sp. nov. and proposal for an emended genus Bacillus limiting it only to the members of the Subtilis and Cereus clades of species. Int J Syst Evol Microbiol 70:5753–5798

Kim OS, Cho YJ, Lee K, Yoon SH, Kim M et al (2012) Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol 62:716–721

Kim I, Chhetri G, Kim J, Seo T (2019) Annibacterium setariae sp. nov., an endophytic actinobacterium isolated from dried foxtail. Antonie Van Leeuwenhoek 112:1731–1738

Kim H, Chhetri G, Kim J, Kang M, Seo T (2020a) Lewinella aurantiaca sp. nov., a carotenoid pigment-producing bacterium isolated from surface seawater. Int J Syst Evol Microbiol 70:6180–6187

Kim J, Chhetri G, Kim I, Kim H et al (2020b) Methylobacterium terrae sp. nov., a radiation-resistant bacterium isolated from gamma ray-irradiated soil. J Microbiol 959:966

Komagata K, Suzuki KI (1987) Lipid and cell-wall analysis in bacterial systematics. Methods Microbiol 19:161–205

Kumar S, Stecher G, Tamura K (2016) Mega7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874

Kuykendall LD, Roy MA, O’Neill JJ, Devine TE (1988) Fatty acids, antibiotic resistance and deoxyribonucleic acid homology groups of Bradyrhizobium japonicum. Int J Syst Evol Microbiol 38:358–361

Logan NA, Lebbe L, Verhelst A, Goris J et al (2002) Bacillus luciférens sp. nov., from volcanic soil on Candlemas Island, South Sandwich archipelago. Int J Syst Evol Microbiol 52:1985–1989

Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinform 14:60

Minnikin DE, O’Donnell AG, Goodfellow M, Alderson G, Athalye M et al (1984) An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. J Microbiol Methods 2:233–241

Na SL, Kim YO, Yoon SH, Ha SM, Baek I et al (2018) UBCG: up-to-date bacterial core gene set and pipeline for phylogenomic tree reconstruction. J Microbiol 56:280–285

Pan T, He H, Wang X, Shen Y, Zhao J, Yan K et al (2017) Bacillus solisilvae sp. nov., isolated from forest soil. Int J Syst Evol Microbiol 67:4449–4455

Peak KK, Duncan KE, Véguiilla W, Luna VA, King DS et al (2007) Bacillus acidiclea sp. nov., isolated from a forensic specimen, containing Bacillus anthracis pX02 genes. Int J Syst Evol Microbiol 57:2031–2036

Rai M, Varma A (2005) Arbuscular Mycorrhiza-like biotechnological potential of Pifirormospora indica, which promotes the growth of Adhatoda Vasica Nees. Electron J Biotechnol 8:1–6

Rzhetsky A, Nei M (1992) A simple method for estimating and testing minimum-evolution trees. Mol Biol Evol 9:945–967

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Smibert RM, Krieg NR (1994) Phenotypic Characterization. In: Gerhardt P, Murray RGE, Wood WA, Krieg NR (eds) Methods for general and molecular bacteriology. American Society for Microbiology, Washington, pp 607–654

Titusova T, DiCuccio M, Badretnin A, Chetvernin V, Nawrocki EP et al (2016) NCBI prokaryotic genome annotation pipeline. Nucleic Acids Res 44:6614–6624

Thompson J, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882

Vandeputte O, Oden S, Mol A, Vereecke D, Goethals K et al (2005) Biosynthesis of Auxin by the gram-positive Phytopathogen Rhodococcus fascians is controlled by compounds specific to infected plant tissues. Appl Environ Microbiol 71:1169–1177

Xu L, Dong Z, Fang L, Luo Y, Wei Z et al (2019) OrthoVenn2: a web server for whole-genome comparison and annotation of orthologous clusters across multiple species. Nucleic Acids Res 47:W52–W58

Yoon SH, Ha SM, Lim J, Kwon S, Chun J (2017) A large-scale evaluation of algorithms to calculate average nucleotide identity. Antonie Van Leeuwenhoek 110:1281–1286

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