Gottfriedia Endophyticus sp. nov., a Novel Indole-Acetic Acid Producing Bacterium Isolated From The Roots of Rice Plant

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Research Article

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Abstract

A Gram-stain-positive, aerobic, motile and rod-shaped bacterium, designated RG28^T, was isolated from the roots of rice plant collected from paddy fields in Goyang, South Korea. Cells of the strain were oxidase-negative but catalase-positive. Strain RG28^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 $C_{16:0}$, iso-$C_{15:0}$ and anteiso-$C_{15:0.}$ The major polar lipids included phosphatidylethanolamine, diphosphatidylglycerol, 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^T (98.6%), *Gottfriedia solisilvae* LMG 18422^T (98.5%) and *Gottfriedia luciferensis* LMG 18422^T (98.4%). The average nucleotide identity (ANI) and in silico DNA–DNA hybridization (isDDH) values between strain RG28^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^T represented a novel species of the genus *Gottfriedia*, for which the name *Gottfriedia endophyticus* sp. nov. is proposed, with the type strain RG28^T (=KCTC 43327^T=TBRC 15151^T).

Repositories: The draft genome and 16S rRNA gene sequences of strain RG28^T have been deposited in GenBank/EMBL/DDBJ under accession numbers JAGIYQ000000000 and MW386408 respectively.

Introduction

The genus *Bacillus*, containing 293 species and subspecies, was recently reclassified on the basis of phylogenomic and comparative genomic frameworks, and six genera, *Cytobacillus*, *Peribacillus*, *Mesobacillus*, *MetabacillusNeobacillus*, *Metabacillus* and *Alkalihalobacillus*, were separated from it, limiting *Bacillus* to only the members of the Subtilis and Cereus clades of species (Gupta et al. 2020). Members of *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). The type species for this genus is *Gottfriedia luciferensis* (Logan et al. 2002). The genus *Gottfriedia* contains only three species with validly published names (https://lpsn.dsmz.de/genus/gottfriedia). Species of *Gottfriedia* have been isolated from soils and forensic specimen so far (Logan et al. 2002; Pan et al. 2017; Peak et al. 2007). 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. 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 RG28^T and other three close strains in the genus *Gottfriedia*.

Materials And Methods

Isolation and ecology

Samples from paddy field were collected near Dongguk University, Goyang, 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; Chhetri et al. 2020; Chhetri et al. 2021a; Chhetri et al. 2021b). 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 (Difco) at 28°C for 1 week. A single colony chosen on the plates was purified by transferring to new R2A plates. Purified colonies were sent to Bionics (Daejeon, Republic of Korea) for 16S rRNA gene analysis. From the purified bacterial colonies, a novel strain of the genus was identified to be a member of *Gottfriedia* and was designated as RG28^T. 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. 2020). 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 at al. 2012) was used to identify strain RG28^T, 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 RG28^T 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 RG28^T 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 RG28^T 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 RG28^T 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), marine agar (MA), nutrient agar (NA), tryptic soy agar (TSA) and Luria-bertani agar (LB) at 30˚C for seven days. The pH range for growth was determined by

cultivation at 30°C in R2A broth adjusted to pH 4-10 (at pH 1 unit intervals) before sterilization with citrate/NaH_2PO4 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. 2020). 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 RG28^T 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 (bioMérieux) system.

Chemotaxonomic analysis

The cellular fatty acid profiles of strains RG28^T 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 vaccum. The sample was re-extracted with acetone and analysed by HPLC as described previously (Kuykendall et al. 1988). For peptidoglycan analysis, cells of strain RG28^T 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 RG28^T 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 1ml was mixed with 2ml of Salkowski's reagent (2% 0.5 FeCl3 in 35% HCLO4 solution), then incubated at room temperature for 30 minutes. 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 RG28^T, which has been stored in the GenBank databases under the accession number MW386408. The full length of the 16S rRNA gene of strain RG28$^{T\&}$ showed the highest sequence similarity to *G. acidiceleris* CBD 119$^{T\&}$ (98.6%), *G. solisilvae* LMG 18422^T (98.5%) and *G. luciferensis* LMG 18422^T (98.4%). Sequence similarities to all other species of the genus *Bacillus*, *Cytobacillus*, *Neobacillus*, *Sutcliffiella* and *Metabacillus* were below 95.2%. The NJ phylogenetic analysis revealed that strain RG28^T formed a separate cluster with *G. acidiceleris* CBD 119^T, *G. solisilvae* LMG 18422^T and *G. luciferensis* LMG 18422^T 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 RG28^T did not vary with the tree reconstruction method used. Reference strains *Gottfriedia acidiceleris* KEMB 1602-188^T, *G. luciferensis* KEMB 7305-013^T and *G. solisilvae* DSM 100485^T 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 RG28^T 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 3,940 genes were predicted with 3,802 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 RG28^T and& *G. acidiceleris* KEMB 1602-188^T, *G. solisilvae* DSM 100485^T and *G. luciferensis* KEMB 7305-013^T 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 RG28^T and& *G. acidiceleris* KEMB 1602-188^T, *G. solisilvae* DSM 100485^T and *G. luciferensis* KEMB 7305-013^T were 21.6, 21.8 and 21.9%, respectively, which was well below the 70% threshold described by Chun *et al.* (2018). These results indicated that strain RG28^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^T and& *G. acidiceleris* KEMB 1602-188^T, 77 between strain RG28^T and& *G. solisilvae* DSM 100485^T and 71 between strain RG28^T and& *G. luciferensis* KEMB 7305-013^T.& According to the genome-based phylogeny, strain RG28^T formed a clade with& *G. acidiceleris* KEMB 1602-188^T, *G. solisilvae* DSM 100485^T and *G. luciferensis* KEMB 7305-013^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^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^T and its closest relatives, the gene cluster for thiopeptide was only found in strain RG28^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^T (Table S1), which is consistent with our results showing the production of endospores.& The genome of strain RG28^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 (JAGIYQ010000021)& which indicates that strain RG28^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^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^T (Table 2). These genomic features indicate that strain RG28^T and its reference strains could be a PGPR candidate.

Phenotypic features

Strain RG28^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 occured 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 RG28^T grew well on R2A, NA, TSA and LB but grew only moderately on MA. Strain RG28^T was tolerant up to 5% NaCl, while& *G. solisilvae* LMG 18422^T tolerated to 4%,& *G. acidiceleris* CBD 119$^{T&}$ to 2 % and& *G. luciferensis* LMG 18422^T 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 RG28^T from its close strains. The detailed physiological and biochemical characteristics of strain RG28^T are summarized in the species description and characteristics that differentiated strain RG28^T from its closest related type strains are listed in Table 1.

Chemotaxonomic analysis

The whole-cell fatty acid profile of strain RG28^T contained large amounts of $C_{16:0}$ (25.5%), iso-$C_{15:0\&}$ (26.4%) and anteiso-$C_{15:0\&}$ (33.4%).& A comparison of strain RG28^T with closely related members of the genus& *Gottfriedia* is presented in Table S2. Strain RG28^T had a similar fatty acid profile to& *G. acidiceleris* KEMB 1602-188^T,& *G. solisilvae* DSM 100485^T and& *G. luciferensis* KEMB 7305-013^T, but the absence of $C_{18:0\&}$ and iso-$C_{13:0\&}$ in strain RG28^T distinguish it from its close relatives. The peptidodoglycan of strain RG28^T 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 RG28^T as found in all known members of the genus& *Gottfriedia.&* However, strain RG28^T could be distinguished from the reference strains with the proportions of MK-7 and MK-6. In addition,& *G. acidiceleris* CBD 119^T has MK-8, which is not found in strain RG28^T and other two reference strains, which clearly differentiates them from each other (Table 2).& The polar lipid profile of strain RG28^T consist of diphosphatidylglycerol (DPG), phosphatidylethanolamine (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 RG28^T from other closely related strains (Pan et al. 2017).

Indole acetic acid (IAA) production and quantification

Change in color showed that strain RG28^T and its reference strains *G. acidiceleris* KEMB 1602-188^T, *G. solisilvae* DSM 100485^T and *G. luciferensis* KEMB 7305-013^T 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^T 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^T formed a different cluster with the three members of genus& *Gottfriedia&* with high topology. Based on the above polyphasic taxonomic analysis, strain RG28^T 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 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, 30°C) and pH of 5.0–10.0 (optimum, pH 7.0) and tolerates upto 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, naphtol-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 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 acid (meso-DAP) are the major respiratory quinone and the diagnostic amino acid of the cell-wall peptidoglycan, respectively.& The main components of the whole-cell fatty acids (10 %) are& $C_{16:0}$, iso-$C_{15:0}$ and anteiso-$C_{15:0.&}$ The major polar lipids consisted of phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, four unidentified aminophosphoglycolipids, four unidentified aminophospholipids, two unidentified glycolipids, one unidentified aminoglycolipid and four unidentified lipids.& & & & & &

The type strain is RG28$^{T&}$ (=KCTC 43327^T=TBRC 15151^T) which was isolated from the roots of rice plant collected from Goyang, 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 JAGIYQ000000000.&

Abbreviations

ANI, average nucleotide identity; NJ, neighbour joining; ML, maximum-likelihood; ME, minimum-evolution; MP, maximum-parsimony; KEMB, Korean Environmental Microorganisms Bank; DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen; IAA, indole acetic acid.

Declarations

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Conflicts of interest

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

Authors' contributions

GC isolated the bacterium, designed the study, performed the phenotypic and biochemical characterization, and wrote the original draft; IK helped with the analysis of taxonomic data; TS designed and supervised the study, and edited the original draft.

Ethics approval

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

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Tables

Table 1. Presence of genes associated with IAA and siderophore in genomes of strain RG28^T and its reference strains.

Proteins	1	2	3	4
Tryptophan				
Tryptophan synthase subunit beta trpB	J5Y03_RS14090	B6K90_RS09040	CAB05_RS14505	B7R67_RS11880
Tryptophan–tRNA ligase trpS	J5Y03_RS05225	B6K90_RS07255	CAB05_RS11475	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_RS14755	-	-
Tryptophan-rich sensory protein	-	B6K90_RS15130	CAB05_RS10035	B7R67_RS00150
	-			B7R67_RS21420
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
		B6K90_RS19730	CAB05_RS10175	

Strain: 1. RG28^T; 2, *G. acidiceleris* KEMB 1602-188^T; 3, *G. solisilvae* DSM 100485^T; 4, *G. luciferensis* KEMB 7305-013^T.

Table 2. Physiological and biochemical characteristics of strain RG28^T and closely related type strains of the genus *Gottfriedia.*

Characteristics	1	2	3	4
Isolation source	Roots	Forensic speciemen	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	0-3	0-4	0-3
Hydrolysis of :				
Gelatin	-	+	-	+
Urea	-	+	-	-
Casein	+	+	-	+
Starch	+	-	+	+
CM-cellulose	+	-	-	+
Tween 80	+	-	+	+
Enzyme activity:				
Esterase Lipase (C8)	+	-	-	+
Lipase (C14)	-	+	+	-
Valline 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				
$_L$-arabinose	-	+	+	+
$_D$-xylose	-	+	-	-
$_L$-xylose	-	+	-	-
$_D$-glucose	+	-	-	+
$_L$-rhamnose	-	+	+	+
Inositol	+	-	-	+
Methyl-$_D$-mannopyranoside	-	+	-	-
$_D$-trehalose	-	+	+	+
Glycogen	-	+	+	-
Xylitol	-	+	+	+
$_D$-turanose	+	+	-	-
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. RG28^T; 2, G. acidiceleris KEMB 1602-188^T; 3, G. solisilvae DSM 100485^T; 4, G. luciferensis KEMB 7305-013^T. 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.*

Figures

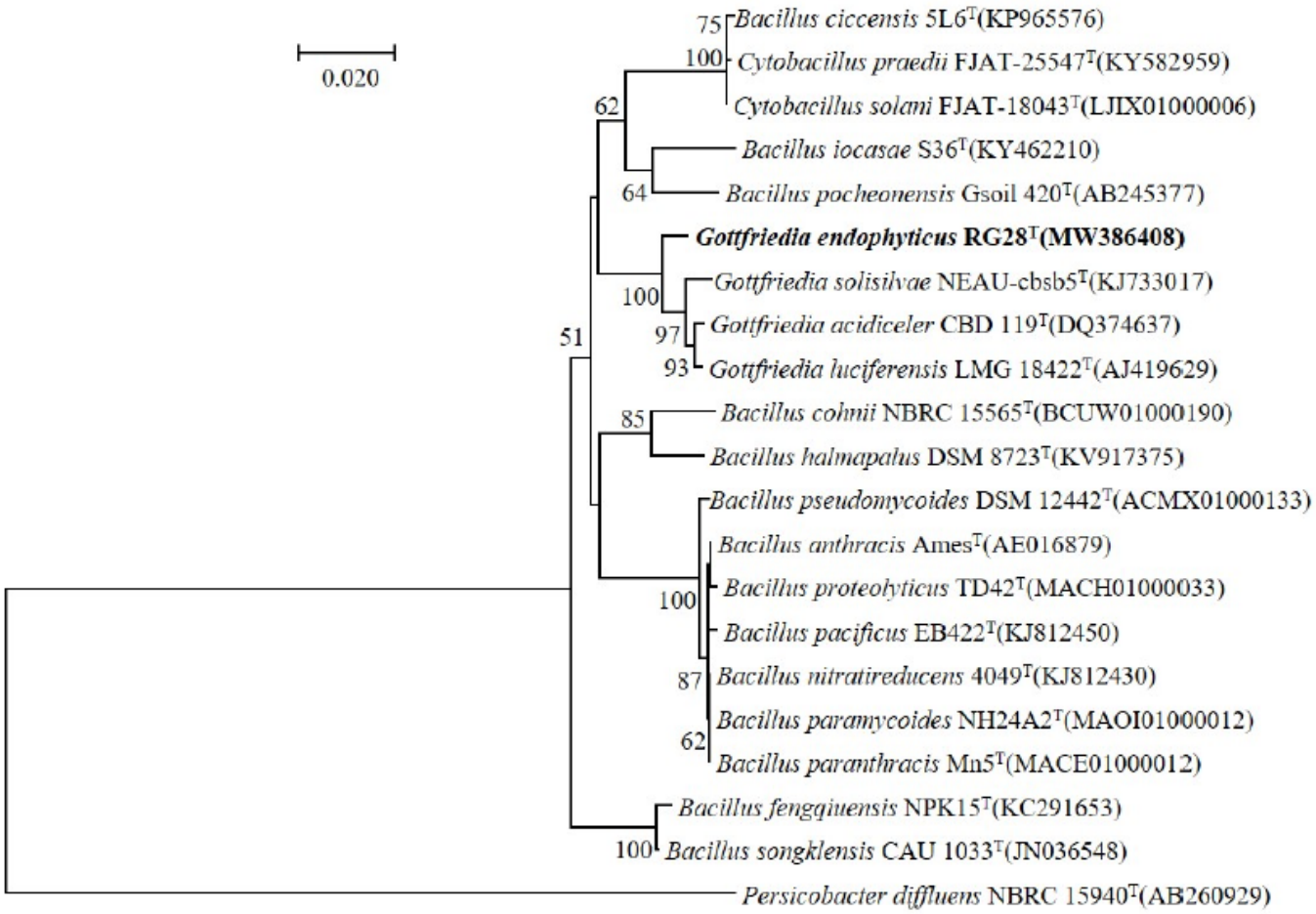

Figure 1

Neighbour-joining tree based on 16S rRNA gene sequences showing the relationship between strain RG28^T and related species. *Persicobacter diffluens* NBRC 15940^T(AB260929) was used as an out-group. Bootstrap values (based on 1000 replications) greater than 50% are shown at branch points. Bar, 0.020 substitutions per nucleotide position.

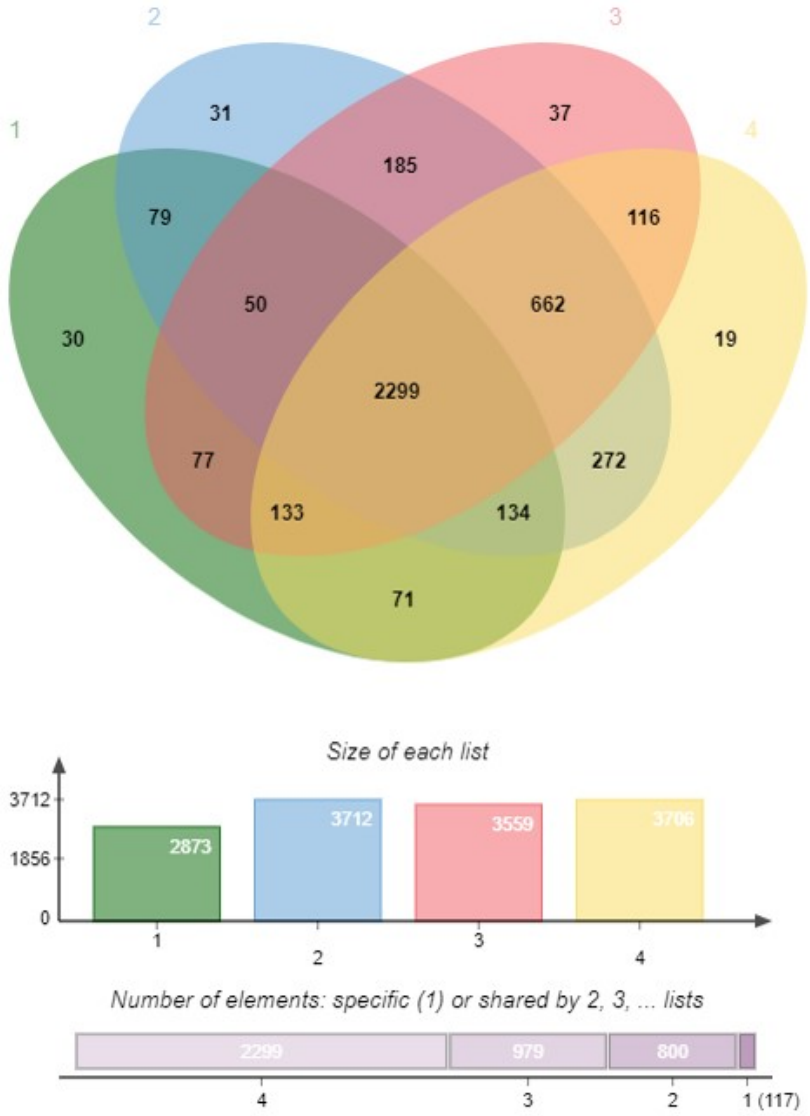

Figure 2

Venn diagram of whole-genome orthologous genes in RG28^{T} and three reference strains. The numbers in the diagram indicate overlapped conserved genes or non-overlapped unique genes in each species. Strain: 1, RG28^{T}; 2, *G. acidiceleris* KEMB 1602-188^{T}; 3, *G. solisilvae* DSM 100485^{T}; 4, *G. luciferensis* KEMB 7305-013^{T}.

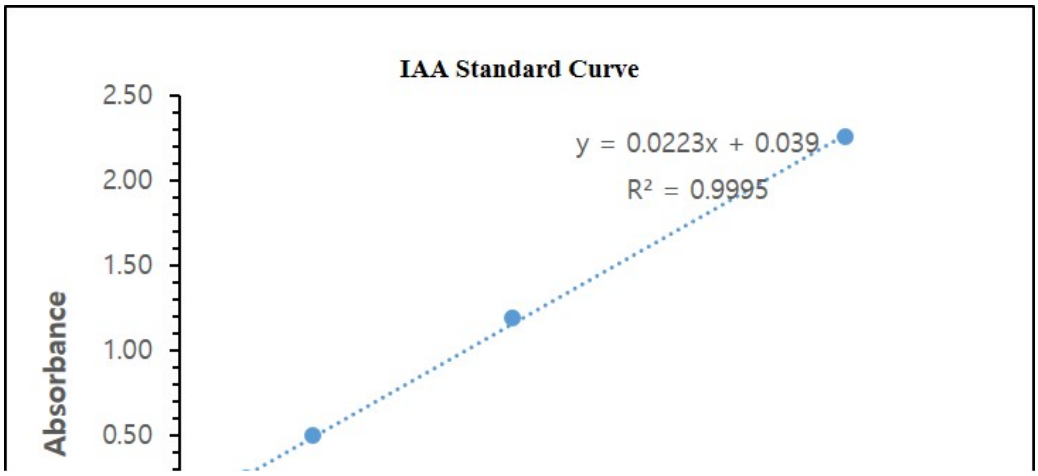

Figure 3

Standard curve and graph of the test results on the production of IAA. All strains were grown on R2A media amended with 0.1% of $_{L}$-tryptophan. Strain: 1, Control (in the absence of bacteria); 2, RG28^{T}; 3, *G. acidiceleris* KEMB 1602-188^{T}; 4, *G. solisilvae* DSM 100485^{T}; 5, *G. luciferensis* KEMB 7305-013^{T}.

Supplementary Files

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