Genome Mining Revealed Polyhydroxybutyrate Biosynthesis by *Ramlibacter Agri* Sp. Nov., Isolated from Agriculture Soil in Korea

Ram Hari Dahal
Kyonggi University
Jungmin Kim
Kyungpook National University
Dhiraj Kumar Chaudhary
Korea University Sejong Campus
Dong-Uk Kim
Sangji UniversitySemyung University
Hyein Jang
Semyung University
Jaisoo Kim (jkimtamu@kgu.ac.kr)
Kyonggi University https://orcid.org/0000-0002-3876-3922

Research Article

Keywords: Ramlibacter agri, agriculture soil, polyhydroxybutyrate, biodegradable plastics, PHB biosynthesis

Posted Date: December 10th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1098143/v1

Version of Record: A version of this preprint was published at Antonie van Leeuwenhoek on March 19th, 2022. See the published version at https://doi.org/10.1007/s10482-022-01721-z.

Abstract

A white-colony-forming, aerobic, motile and Gram-stain-negative bacterium, designated G-1-2-2^T was isolated from soil of agriculture field near Kyonggi University, Republic of Korea. Strain G-1-2-2^T synthesize the polyhydroxybutyrate and could grow at 10–35°C. The phylogenetic analysis of its 16S rRNA gene sequence, strain G-1-2-2^T formed a lineage within the family *Comamonadaceae* and clustered as a member of the genus *Ramlibacter*. The 16S rRNA gene sequence of strain G-1-2-2^T showed high sequence similarities with *Ramlibacter ginsenosidimutans* BXN5-27^T (97.9%), *Ramlibacter monticola* G-3-2^T (97.9%) and *Ramlibacter alkalitolerans* CJ661^T (97.4%). The sole respiratory quinone was ubiquinone-8 (Q-8). The major polar lipids were phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, and an unidentified phospholipid. The principal cellular fatty acids were $C_{16:0}$, cyclo-$C_{17:0}$, summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$) and summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$). The genome of strain G-1-2-2^T was 7,200,642 bp long with 13 contigs, 6,647 protein-coding genes, and DNA G+C content of 68.9%. The average nucleotide identity and *in silico* DNA–DNA hybridization values between strain G-1-2-2^T and closest members were ≤81.2 and 24.1%, respectively. The genome of strain G-1-2-2^T showed eight putative biosynthetic gene clusters responsible for various secondary metabolites. Genome mining revealed the presence of *atoB*, *atoB2*, *phaS*, *phbB*, *phbC*, *bhbD* genes in the genome which are responsible for polyhydroxybutyrate biosynthesis. Based on these data, strain G-1-2-2^T represents a novel species in the genus *Ramlibacter*, for which the name *Ramlibacter agri* sp. nov. is proposed. The type strain is G-1-2-2^T (= KACC 21616^T = NBRC 114389^T).

Introduction

The genus *Ramlibacter* was first proposed by Hulin *et al*. with the description of cyst-producing bacteria *Ramlibacter tataouinensis* and *Ramlibacter henchirensis* isolated from subdesert soil (Heulin et al. 2003). Later on, the genus description has been emended by Lee *et al*. regarding colony colour, extracellular polymeric substance production, motility and aesculin hydrolysis (Lee et al. 2014). To date, 11 species of the genus *Ramlibacter* with validly published names have been reported (https://lpsn.dsmz.de/genus/ramlibacter; accessed date: 2021.11.11) (Parte et al. 2020). Members of the genus *Ramlibacter* are characterized by aerobic, Gram-stain-negative, rod- to coccoid-shaped, motile or non-motile and contain ubiquinone-8 (Q-8) as a predominant isoprenoid quinone; $C_{16:0}$, cyclo-$C_{17:0}$, and summed feature 3 ($C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$) as principal fatty acids; phosphatidylethanolamine, diphosphatidylglycerol and phosphatidylglycerol as major polar lipids (Heulin et al. 2003; Lee et al. 2014; Chaudhary and Kim 2017). Most of the members of the genus *Ramlibacter* have been isolated from soil in addition to few members from aquatic niches (Props et al. 2019; Kim et al. 2021).

In this study, a novel member of the genus *Ramlibacter* isolated from soil of agriculture field near Kyonggi University and its taxonomic position was determined during the study of diversity of soil microorganisms. Moreover, whole-genome analysis of strain G-1-2-2^T has been explored providing insights into polyhydroxybutyrate biosynthesis.

Materials And Methods

Ecology, isolation and preservation

Strain G-1-2-2^T was isolated from soil of agriculture field, geographically located near Kyonggi University, Suwon, Republic of Korea (37°17'56.9"N and 127°02'23.2"E) and subjected to polyphasic approach for its taxonomic description. A modified culture method with six-well Transwell plates (Corning Inc., NY, USA) was utilized for isolation. Sieved 3 g soil was kept on the bottom of each Transwell plate and 3 ml Reasoner's 2A (R2A) broth (Kisan Bio, Seoul, Korea) was added to each insert. Then, 100 µl soil suspension (1 g soil in 9 ml distilled water; thoroughly stirred and settled) was added to the insert. The Transwell plate was incubated in a shaker at 120 r.p.m. for 4 weeks at 28°C. After 4 weeks, the culture was serially diluted, and 100 µl of each dilution was spread on R2A agar plates. Isolation, maintenance and preservation of strain G-1-2-2^T was done as described in previous study (Dahal et al. 2018).

Phylogenetic analysis

Genomic DNA of strain G-1-2-2^T was isolated by using InstaGene Matrix kit (Life Science Research; Bio-Rad, CA, USA) following manufacturer's instruction. The 16S rRNA gene was amplified by using PCR (Bio-Rad, CA, USA) with forward and reverse primers 27F and 1492R, respectively (Frank et al. 2008). Applied Biosystems 3770XL DNA analyzer was used with a BigDye Terminator cycle sequencing Kit v.3.1 (Applied Biosystems, CA, USA) for gene sequencing. After sequencing, nearly complete sequence of 16S rRNA genes was assembled using SeqMan software (DNASTAR Inc., WI, USA). Phylogenetically closest neighbours were identified using the EzBioCloud server (Yoon et al. 2017b). All the 16S rRNA gene sequences of phylogenetically closest neighbours were retrieved from the EzBioCloud server (https://www.ezbiocloud.net/identify) and ncbi GenBank database (https://www.ncbi.nlm.nih.gov/nucleotide). All the retrieved sequences along with the sequence of strain G-1-2-2^T were aligned using *in silico* by sina alignment (https://www.arb-silva.de/aligner) (Pruesse et al. 2012). Neighbour-joining (NJ), maximum-likelihood (ML) and maximum-parsimony (MP) phylogenetic trees were reconstructed using mega v7.0.26 software (Kumar et al. 2016).

Reference strains

Based on the 16S rRNA gene sequence similarities and phylogenetic analyses *Ramlibacter ginsenosidimutans* KACC 17527^T; 3, *R. monticola* KACC 19175^T; 4, *R. alkalitolerans* KACC 19305^T were used as references and analysed under identical conditions for API, biochemical and fatty acid analyses.

Genome analyses

For genome sequencing, extraction of genomic DNA was carried out by using DNeasy Blood and Tissue kits (Qiagen). Whole-genome shotgun sequencing of strain G-1-2-2^T was performed at Macrogen (Republic of Korea) using the Illumina HiSeq 2500 platform using a 150-bp × 2 paired-end kit. The whole-genome sequences were assembled by SPAdes v3.2 (Bankevich et al. 2012). The authenticity of the assembled genome was analysed by comparing 16S rRNA gene sequence of strain G-1-2-2^T using ncbi Basic Local Alignment Search Tool (blastn) (Zhang et al. 2000). Potential contamination of genome assembly was

examined *in silico* by ContEst16S algorithm using EzBioCloud server (https://www.ezbiocloud.net/tools/contest16s) (Lee et al. 2017). Then, the whole-genome sequence (WGS) of strain G-1-2-2^T was annotated using the Rapid Annotation using Subsystem Technology (RAST; https://rast.nmpdr.org) server (Aziz et al. 2008) and Prokaryotic Genome Annotation Pipeline (PGAP; https://www.ncbi.nlm.nih.gov/genome/annotation_prok) (Tatusova et al. 2016). All the genome sequences of reference strains were retrieved from ncbi database. The DNA G+C content of strain G-1-2-2^T and other references used in this study were calculated based on respective whole-genome sequences. Genome-based relatedness between G-1-2-2^T and phylogenetically closest neighbours were determined based on ANI (Average Nucleotide Identity) *in silico* by OrthoANIu (https://www.ezbiocloud.net/tools/ani) algorithm (Yoon et al. 2017a). The phylogenomic tree was reconstructed *in silico* using concatenated alignment of 92 core genes with UBCGs software (Na et al. 2018). Digital DNA-DNA hybridization (dDDH) was calculated *in silico* by the Genome-to-Genome Distance Calculator (GGDC 3.0) using the blast method (https://ggdc.dsmz.de/ggdc.php#) (Meier-Kolthoff et al. 2021). Graphical circular map was constructed by using CGView (http://cgview.ca) server (Grant and Stothard 2008). Transfer RNAs (tRNAs) and ribosomal RNAs (rRNAs) were analysed using tRNAscan-SE (http://lowelab.ucsc.edu/tRNAscan-SE) (Schattner et al. 2005) and rnammer (http://www.cbs.dtu.dk/services/RNAmmer) (Lagesen et al. 2007) servers. The CRISPR genes and Cas clusters were determined *in silico* using the CRISPRCasFinder (https://crisprcas.i2bc.paris-saclay.fr) server. The antiSMASH server (https://antismash.secondarymetabolites.org/#!/start) was used to identify the biosynthetic gene clusters (BGCs) for various secondary metabolites (Blin et al. 2019).

Morphological, biochemical and chemotaxonomic analyses

The cell morphology of strain G-1-2-2^T, grown on R2A agar plate for 4–5 days at 28°C was observed by using TEM (transmission electron microscopy; Talos L120C; FEI). The colony morphology of strain of G-1-2-2^T was observed using a Zoom Stereo Microscope (SZ61; Olympus, Tokyo, Japan). Gram-staining was performed as described previously (Doetsch 1981). The cell motility was examined in R2A (Reasoner's Agar No. 2; MB cell; KisanBio, Seoul, Republic of Korea) soft agar medium consisting 0.4% agar A (Bio Basic; Ontario, Canada). Oxidase and catalase activities of strain G-1-2-2^T was examined using 1% tetra-methyl-*p*-phenylenediamine dihydrochloride and 3% (v/v) H_2O_2, respectively. Growth at various temperatures (0–40°C) on R2A agar plates was observed for 10 days. Growth was monitored on various bacteriological media including brain heart infusion agar (BHI; Oxoid), Luria-Bertani agar (LBA; Oxoid), marine agar 2216 (Becton), nutrient agar (NA; Oxoid), R2A agar, sorbitol MacConkey agar (MA; Oxoid), tryptone soya agar (TSA; Oxoid), and veal infusion agar (VIA; Becton). DNase activity of strain G-1-2-2^T was analysed by using DNase agar (Oxoid). Salt tolerance was checked in R2A broth supplemented with NaCl [Duksan Chemicals, Republic of Korea; 0–5% (w/v) at 0.5% interval]. The pH range was observed at 28°C in R2A broth (pH 4–12 in increments of 0.5 pH units). Hydrolysis of Tween 80, Tween 60 and Tween 40 were analysed as described by Smibert & Krieg (Smibert and Krieg 1994). The anaerobic growth of strain G-1-2-2^T was monitored for 10 days on R2A agar plate at 28°C with BD GasPak^{TM} EZ Gas Generating Pouch System (BD). Hydrolysis of casein CM-cellulose, starch and tyrosine were assessed as described in previous study (Dahal and Kim 2018). Hydrogen sulfide (H_2S) and indole production were checked in SIM sulfide indole motility medium (SIM; Oxoid). Malachite green staining was done to analyse spore. Other physiological tests were examined by using API 20NE and

API ID 32GN kits (bioMérieux). The enzyme activities of strain G-1-2-2^T and other references were examined by using an API ZYM kit (bioMérieux) following the manufacturer's instructions.

For the determination of fatty acids, cells of reference strains and strain G-1-2-2^T were harvested from identical culture condition (at 28°C on R2A agar plate for 4 days). Fatty acid methyl esters (FAME) of harvested cells were extracted using MIDI protocol technical note #101 (http://midi-inc.com/pdf/MIS_Technote_101.pdf). Extracted FAMEs were analysed using a HP 6890 Series GC System (Gas chromatograph; Hewlett Packard; Agilent Technologies) and the FAME compositions (percentage of totals) were identified with TSBA6 database of the Microbial Identification System (Sasser 1990). The polar lipids and isoprenoid quinones were extracted from freeze-dried cells following the protocol of Minnikin *et al*. (Minnikin et al. 1984). Appropriate reagents for the spot detection were used as described by Komagata and Suzuki (Komagata and Suzuki 1988).

Result And Discussions

Phylogenetic analysis

The 16S rRNA gene sequence of strain G-1-2-2^T was 1,483 bp long and has been deposited at GenBank/EMBL/DDBJ database under the accession MN685325. Phylogenetic analysis based on 16S rRNA gene sequence showed that strain G-1-2-2^T formed a lineage within the family *Comamonadaceae* and clustered with members of the genus *Ramlibacter*. The 16S rRNA gene sequence of strain G-1-2-2^T showed high sequence similarities with *Ramlibacter ginsenosidimutans* BXN5-27^T (97.9%), *Ramlibacter monticola* G-3-2^T (97.9%), and *Ramlibacter alkalitolerans* CJ661^T (97.4%). The sequence similarities of strain G-1-2-2^T with all other members of the genus *Ramlibacter* were <96.7%. Strain G-1-2-2^T was clustered with *Ramlibacter alkalitolerans*, *Ramlibacter monticola*, and *Ramlibacter ginsenosidimutans* in all three trees i.e. ML, NJ and MP (Figs. 1, S1 and S2). In addition, in all phylogenetic trees, strain G-1-2-2^T formed a distinct lineage. The phylogenetic positions in all three NJ, ML and MP trees strongly support to assign strain G-1-2-2^T as a novel member within the genus *Ramlibacter*.

Genomic analysis

The ContEst16S analysis of strain G-1-2-2^T showed that the genome was not contaminated. Whole-genome shotgun sequence has been deposited at DDBJ/ENA/GenBank under the accession JABBFX000000000. The genome size and N50 value of strain G-1-2-2^T are 7,200,642 bp and 3,830,216 bp, respectively. The genome has 13 scaffolds and coverage of 139.0-fold (Table S1). The graphical genomic map revealed the presence of six rRNAs and 52 tRNAs (Fig. 2). The DNA G+C content of strain G-1-2-2^T is 68.9% which is within the range of *Ramlibacter* species (Heulin et al. 2003; Lee et al. 2014). The ANI threshold for species demarcation is recommended at 95-96% (Richter and Rosselló-Móra 2009) and ANI values between strain G-1-2-2^T and other *Ramlibacter* members were ≤81.2% (Table 1). The dDDH values of ≤24.1% was much lower value than the species threshold of 70% recommended for species delineation (Meier-Kolthoff et al. 2013) (Table 1). These values clearly show that strain G-1-2-2^T represents a novel member within the genus *Ramlibacter* (Meier-Kolthoff et al. 2013). Furthermore, the phylogenomic position of strain G-1-2-2^T obtained from tree

reconstructed using UBCGs (concatenated alignment of 92 core genes) also showed that strain G-1-2-2^T is a novel member of the genus *Ramlibacter* (Fig. S3).

Table 1
Average nucleotide identity (OrthoANIu) and digital DNA-DNA hybridization (dDDH) between strain G-1-2-2^T and other members of the genus *Ramlibacter*.

Strains	GenBank accessions	G-1-2-2^T	
		ANI	dDDH
Ramlibacter algicola CrO1^T	JAEDAO000000000	78.4	21.6
Ramlibacter alkalitolerans KACC 19305^T	JAEQND000000000	81.0	24.1
Ramlibacter aquaticus LMG 30558^T	JADDOJ000000000	78.8	22.0
Ramlibacter ginsenosidimutans KACC 17527^T	JAEPWM000000000	80.8	24.0
Ramlibacter henchirensis DSM 14656^T	SMLM00000000	78.7	21.6
Ramlibacter humi 18x22-1^T	SMLK00000000	79.1	22.0
Ramlibacter monticola KACC 19175^T	JAEQNE000000000	81.2	24.1
Ramlibacter pinisoli MAH-25^T	WSEL00000000	79.0	22.0
Ramlibacter rhizophilus CCTCC AB2015357^T	SMLL00000000	77.8	21.4
Ramlibacter solisilvae 5-10T^T	CP010951	79.1	22.0
Ramlibacter tataouinensis TTB310^T	CP000245	79.6	22.4

The RAST analysis revealed the presence of 335 subsystems, 70 polyhydroxybutyrate (PHB) metabolism and five secondary metabolisms (alkaloid biosynthesis from L-lysine: one, auxin biosynthesis: four; Fig. S4). The genome of strain G-1-2-2^T consists eight putative BGCs (arylpolyene, RiPP-like, linaridin, T1PKS, NRPS-like, terpene, redox-cofactor, betalactone, and phosphonate) were revealed by antiSMASH analysis (Table 2). The genome contained the genes such as *atoB*, *atoB2*, *phaS*, *phbB*, *phbC*, *bhbD* which are responsible for PHB biosynthesis. In addition, the genome contained genes encoding a *β*-ketothiolase (WP_169422749), and class I poly(R)-hydroxyalkanoic acid synthase (WP_169420190, WP_169422744). These genes are the key enzyme for PHB biosynthesis (Catone et al. 2014). The PHB derived from bacteria not only be used as carbon and energy reserve materials (Muneer et al. 2020) but also could be utilized in making biodegradable plastics (Getachew and Woldesenbet 2016; Mostafa et al. 2020).

Table 2

Numbers of predicted secondary metabolite biosynthetic gene clusters (smBGC) of G-1-2-2^T genome. The BGCs were determined using anti-SMASH (v5.1.2). T1PKS, type I polyketide synthase; NRPS, non-ribosomal peptide synthetase cluster; RiPP-like; other unspecified ribosomally synthesised and post-translationally modified peptide; NRP, non-ribosomal peptide; APE Ec, aryl polyene.

Cluster	Contig	BGC type	From	To	Most similar known cluster	Core biosynthetic gene	Additional biosynthetic gene
1	1	arylpolyene	459,642	503,219	APE Ec, othter (36%)	2	11
2	1	RiPP-like, linaridin	1,132,400	1,155,291	-	2	0
3	1	RiPP-like	1,577,922	1,588,755	-	1	0
4	1	T1PKS, NRPS-like	2,474,608	2,523,951		2	1
5	1	terpene	3,171,214	3,192,922	-	2	2
6	1	redox-cofactor	3,626,740	3,648,809	lankacidin C, NRP + polyketide (13%)	3	1
7	3	betalactone	339,687	370,393	fengycin, NRP (13%)	2	6
8	3	phosphonate	470,992	511,870	-	1	6

Physiological analysis

The cells of strain G-1-2-2^T are rod-shaped (Fig. S5), aerobic, catalase and oxidase positive, non-spore-forming, Gram-stain-negative and motile with flagella. Strain G-1-2-2^T formed while-colony on R2A agar plate and PHB was accumulated in its cells (Fig. S5). Weak growth was observed in anaerobic condition when incubated for 10 days. Strain G-1-2-2^T hydrolysed urea but not CM-cellulose, casein, starch, gelatin, tyrosine, DNA, Tween 80, Tween 60 and Tween 40. The differential physiological characteristics are given on Table 3 along with its closest reference strains.

Table 3. Phenotypic characteristics of strain G-1-2-2^T of the genus *Ramlibacter* that differentiates with phylogenetically related type species.

Strains: 1, G-1-2-2^T; 2, *R. ginsenosidimutans* KACC 17527^T; 3, *R. monticola* KACC 19175^T; 4, *R. alkalitolerans* KACC 19305^T. All data were obtained from this study. +, positive; w, weak; –, negative.

Characteristic	1	2	3	4
Maximum growth temperature (°C)	35	37	37	37
Highest salt tolerance (%, w/v)	1.0	0.5	0.5	0.5
pH range	5.0–11.0	6.0–9.0	5.5–10.0	5.0–10.5
Nitrate reduction	–	+	–	+
Esculin hydrolysis	w	–	w	–
Urease	+	–	+	–
Enzyme activity (API ZYM)				
Acid phosphatase	+	–	+	–
Alkaline phosphatase	+	–	+	–
Cystine arylamidase	–	–	–	+
Esterase (C4)	+	+	–	+
Esterase lipase (C8)	+	–	+	+
Leucine arylamidase	+	–	+	–
Lipase (C14)	–	–	–	+
Napthol-AS-BI-phosphohydrolase	+	–	+	+
Valine arylamidase	+	–	+	+
α-galactosidase	–	–	–	+
β-galactosidase	–	+	–	–
β-glucosidase	–	+	–	–
Assimilation from				
d-glucose	–	+	–	–
l-arabinose	–	–	–	+
d-mannose	–	–	–	+
d-mannitol	–	–	–	+
N-acetyl-glucosamine	–	–	–	+
d-maltose	–	+	–	–
DNA G + C content (%)	68.9	68.7	69.3	69.2

The principal fatty acids of strain G-1-2-2^T were $C_{16:0}$, cyclo-$C_{17:0}$, summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$) and summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$), similar with the genus *Ramlibacter*. However, the differences in major and minor fatty acids in addition to the presence of minor fatty acids such as iso-$C_{10:0}$, iso-$C_{19:0,}$ iso-$C_{8:0}$ 3-OH, and iso-$C_{15:0}$ 3-OH differentiate strain G-1-2-2^T from other closely related type species of the genus *Ramlibacter* (Table S2). The sole respiratory quinone was ubiquinone-8 (Q-8) and the major polar lipids of strain G-1-2-2^T were phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG), phosphatidylglycerol (PG) and an unidentified phospholipid (PL). In addition, one unidentified aminolipid (AL) and three unidentified polar lipids (L1-L3) were also detected in TLC chromatograms (Fig. S6).

Based on above discussed data, strain G-1-2-2^T represents a novel species in the genus *Ramlibacter* for which the name *Ramlibacter agri* sp. nov. is proposed.

Description of Ramlibacter agri **sp. nov.**

Ramlibacter agri (a'gri. L. gen. n. *agri* of an agriculture field, referring to the source of isolation).

Cells (1.5–2.5 μm long and 0.6–0.9 μm wide) are rod-shaped, aerobic, Gram-stain-negative and motile with flagella. Colonies (1–2 mm) are white-coloured, circular and convex on R2A agar plate after 5 days at 28°C. Colonies grow well on R2A agar, weakly on TSA and no growth is observed on NA, BHI, LBA, MA, VIA and marine agar 2216. Colonies grow at 10–35°C (optimum, 25–30°C) and pH 5.0–11.0 (optimum pH, 7.0–8.5). Cells grow optimally in the absence of NaCl but tolerate 1% (w/v) of NaCl. Catalase and oxidase are positive. Hydrogen sulfide is not produced. Nitrate is not reduced to nitrite. Glucose is not fermented. The type strain shows the following enzyme activities: positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase and napthol-AS-BI-phosphohydrolase; and negative for lipase (C14), cystine arylamidase, trypsin, *α*-chymotrypsin, *α*-galactosidase, *β*-galactosidase, *α*-glucosidase, *β*-glucosidase, *N*-acetyl-*β*-glucosaminidase, *β*-glucuronidase, *α*-mannosidase and *α*-fucosidase. l-rhamnose, lactic acid, 3-hydroxybenzoic acid, d-mannitol, l-arabinose, propionic acid, 3-hydroxybutyric acid, 4-hydroxybenzoic acid, and l-proline are assimilated. The sole respiratory quinone is Q-8. The principal cellular fatty acids are $C_{16:0}$, cyclo-$C_{17:0}$, summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$) and summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$). The major polar lipids are phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, and an unidentified phospholipid. The DNA G + C content of the type strain is 68.9%.

The type strain, G-1-2-2^T (= KACC 21616^T = NBRC 114389^T), was isolated from soil of agriculture field, geographically located near Kyonggi University, Suwon, Republic of Korea (37°17'56.9"N and 127°02'23.2"E). The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and the whole genome sequence of strain G-1-2-2^T are MN685325 and JABBFX000000000, respectively.

Declarations

Funding information

This paper was supported by the Semyung University Research Grant of 2020.

Acknowledgements

We thank Prof. Aharon Oren (The Hebrew University of Jerusalem, Israel) for his expert suggestions concerning the correct species epithet and etymology.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Ethical Statement

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

Author contributions

R.H.D., J.K. (2nd), D.K.C. conceived, designed and conducted all the experiments. D.U.K. interpreted the data. J.K. (6th) and H.J. coordinated and supervised the study. R.H.D., J.K. (both), D.K.C. and D.U.K. analysed all the data and prepared the manuscript. All the authors read, discussed, edited and approved the final draft of the manuscript.

Data availability

The genome sequence and 16s rRNA gene sequence are publicly available in NCBI database.

Informed consent

Not applicable

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Figures

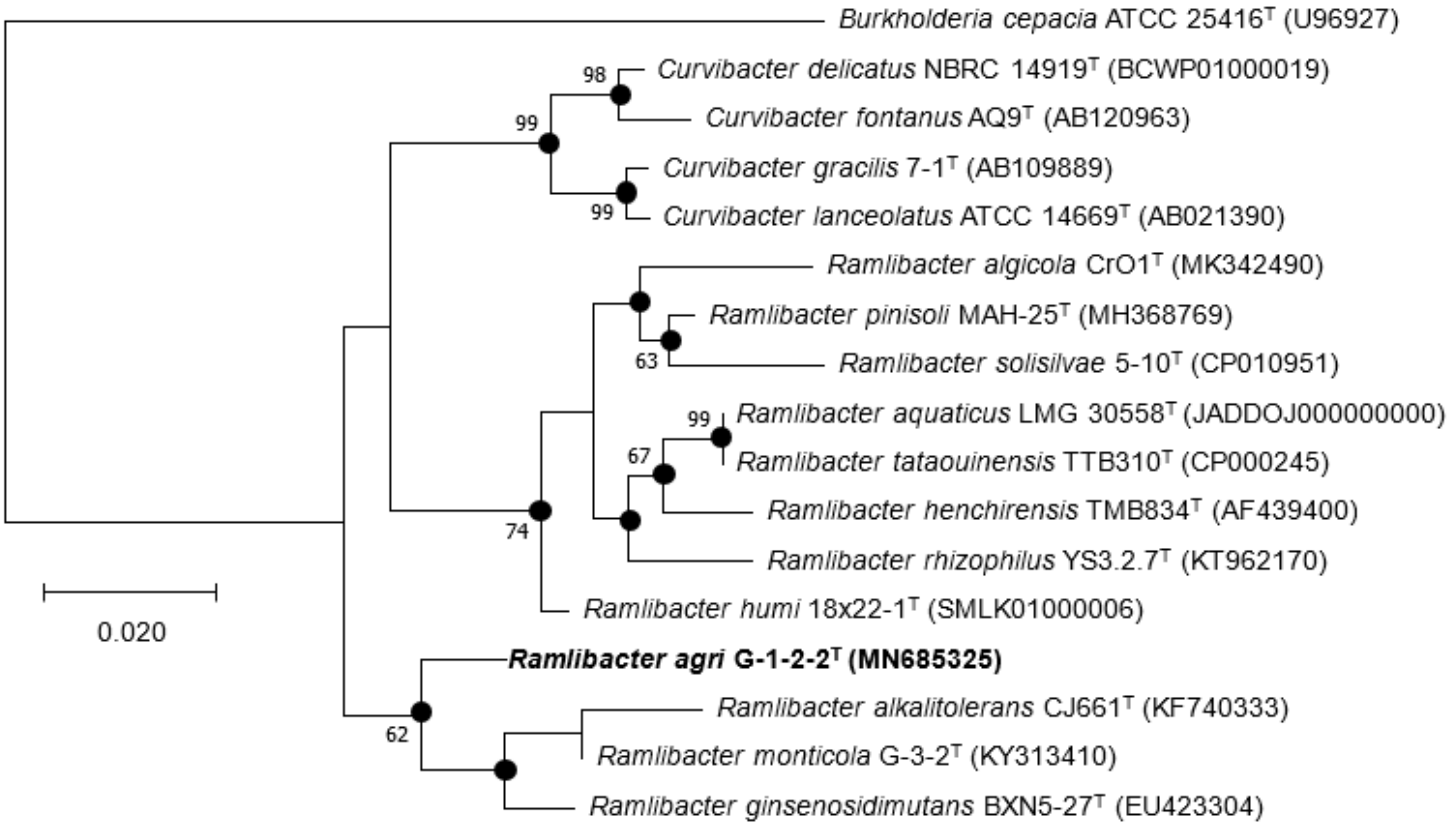

Figure 1

Maximum-likelihood (ML) phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic position of strain G-1-2-2T among the closest members of the family Comamonadaceae. Filled circles are recovered by neighbour–joining (NJ), maximum–likelihood (ML) and maximum-parsimony (MP) treeing methods. Bootstrap values (>50%) based on 1000 bootstrap replicates are shown at branch nodes. Burkholderia cepacia ATCC 25416T was used as an out-group. Numbers in parentheses indicate GenBank accession numbers. Bar, 0.020 substitutions per nucleotide position.

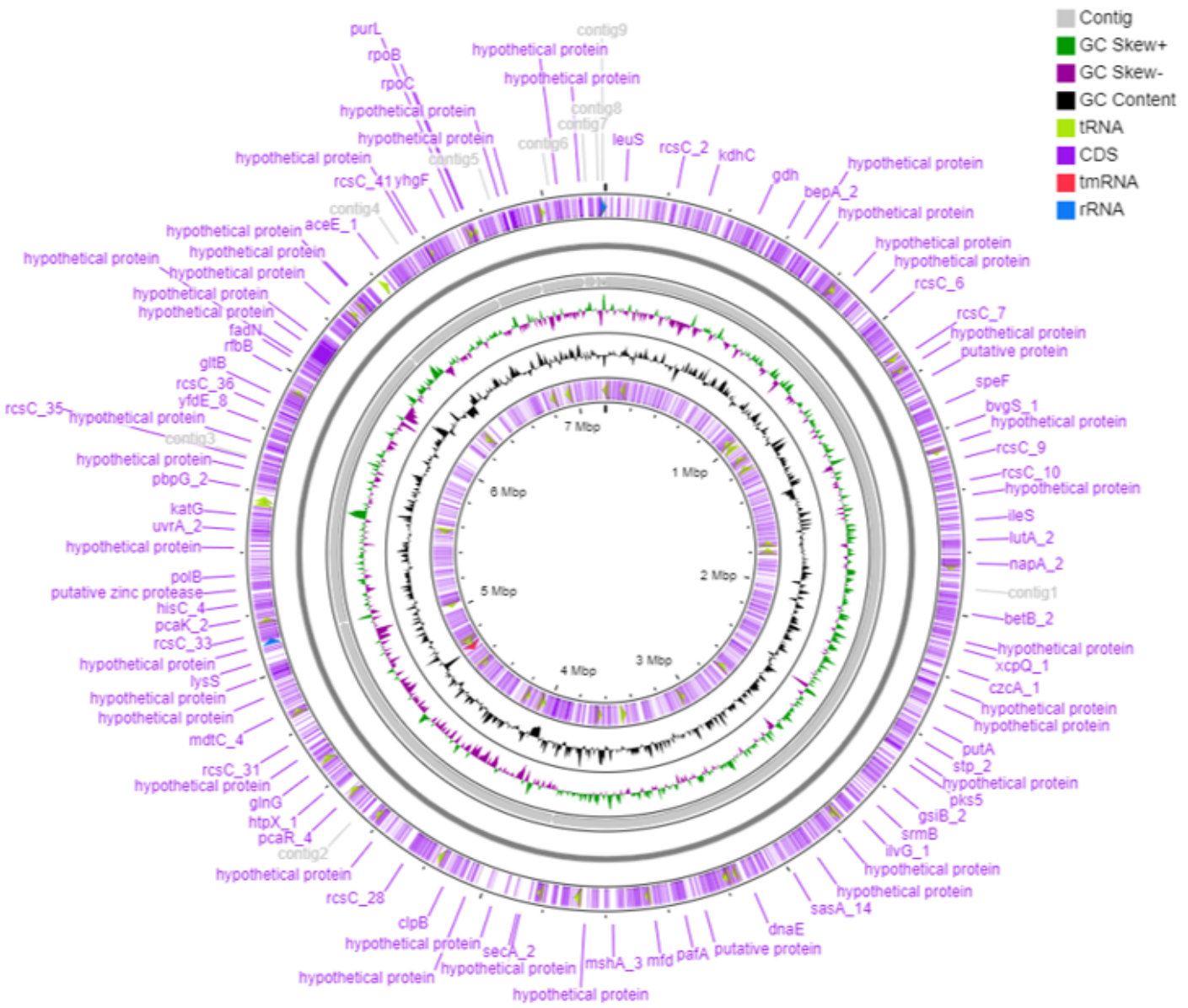

Figure 2

Graphical genomic map of strain G-1-2-2T. From outside to the center: Genes on forward strand, genes on reverse strand, RNA genes (tRNAs purple blue, rRNAs blue, tmRNA red), GC content, and GC skew.

Supplementary Files

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