INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

RESEARCH ARTICLE

Huq *et al.*, *Int. J. Syst. Evol. Microbiol.* 2023;73:005858
DOI 10.1099/ijsem.0.005858

MICROBIOLOGY SOCIETY

Solitalea agri sp. nov., a new member of the genus *Solitalea* isolated from rhizospheric soil of a jujube tree

Md. Amdadul Huq[1,*], Sun-Young Lee[1], BoKyung Moon[1], Changsun Choi[1], Sung-Kwon Moon[1] and Shahina Akter[2,*]

Abstract

A Gram-stain negative, aerobic, rod-shaped and creamy pink-coloured bacterium, designated MAHUQ-68^T, was isolated from rhizospheric soil of a jujube tree. Colonies grew at 10–40 °C (optimum, 28 °C), pH 6.0–9.0 (optimum pH, 7.0) and in the presence of 0–1.5% NaCl (optimum 0–0.5%). Positive for both catalase and oxidase activity. Strain MAHUQ-68^T hydrolysed casein, starch, aesculin and L-tyrosine. Based on the results of phylogenetic analysis using 16S rRNA gene and genome sequences, strain MAHUQ-68^T clustered together within the genus *Solitalea*. The closest members were *Solitalea longa* HR-AV^T (98.8% sequence similarity), *Solitalea canadensis* DSM 3403^T (96.9%) and *Solitalea koreensis* R2A36-4^T (94.0%). The genome of strain MAHUQ-68 ^{T} was 4250173 bp long with 68 scaffolds and 3570 protein-coding genes. The genomic DNA G+C content of the type strain was 38.0 mol%. The average nucleotide identity and *in silico* DNA–DNA hybridization values between strain MAHUQ-68^T and its closest relatives were 72.0–81.4% and 19.8–24.3%, respectively. The major cellular fatty acids were iso-$C_{15:0}$ and summed feature 3 ($C_{16:1}$ ω7*c* and/or $C_{16:1}$ ω6*c*). The main respiratory quinone was menaquinone-7. The polar lipids comprised phosphatidylethanolamine, an unidentified aminolipid and four unidentified lipids. Based on these data, strain MAHUQ-68^T represents a novel species in the genus *Solitalea*, for which the name *Solitalea agri* sp. nov. is proposed. The type strain is MAHUQ-68^T (=KACC 22249^T=CGMCC 1.19062^T).

INTRODUCTION

The genus *Solitalea* was first proposed by Weon *et al.* [1] with the description of *Solitalea koreensis* isolated from greenhouse soil, belonging to the family *Sphingobacteriaceae* [2] of the phylum *Bacteroidota* [3]. At the time of writing, the genus *Solitalea* comprises only three species, *Solitalea longa*, *Solitalea koreensis* and *Solitalea canadensis* (https://lpsn.dsmz.de/genus/solitalea). The type species of the genus, *S. koreensis*, was isolated from greenhouse soil [1], *S. longa* was isolated from freshwater [4] and *S. canadensis* was isolated from a soil sample [5]. *S. canadensis* was formerly known as *Flexibacter canadensis* [5], and subsequently transferred into the genus S*olitalea* by Weon *et al.* [1] as *S. canadensis* on the basis of phylogenetic and chemotaxonomic data. Members of the genus *Solitalea* are characterized by Gram stain-negative, strictly aerobic or facultative anaerobic, catalase and oxidase positive, elongated rods or non-flagellated rods. Members of this genus contain menaquinone-7 (MK-7) as the major isoprenoid quinone and iso-$C_{15:0}$ and summed feature 3 ($C_{16:1}$ ω7*c* and/or $C_{16:1}$ ω6*c*) as predominant fatty acids. The genomic DNA G+C contents of this genus are between 37.3 and 38.4 mol% [1, 4]. In this study, a novel species of the genus *Solitalea* was isolated from rhizosperic soil of the jujube plant during the investigation of bacterial diversity in a jujube garden and its taxonomic position was determined using a polyphasic approach. To clarify the taxonomic position of strain MAHUQ-68^T, we have investigated different physiological, biochemical, chemotaxonomic and genotypic characteristics along with reference strains. On the basis of the data acquired during this study, we propose that strain MAHUQ-68^T should be placed in the genus *Solitalea* as the type strain of novel species, namely *Solitalea agri* sp. nov.

Author affiliations: [1]Department of Food and Nutrition, College of Biotechnology and Natural Resource, Chung-Ang University, Anseong-si, Gyeonggi-do, 17546, Republic of Korea; [2]Department of Food Science and Biotechnology, Gachon University, Seongnam, 461-701, Republic of Korea.
***Correspondence:** Md. Amdadul Huq, amdadbge@gmail.com;amdadbge100@cau.ac.kr; Shahina Akter, shahinabristy16@gmail.com
Keywords: *Solitalea agri*; rhizospheric soil; genome sequence; DNA–DNA hybridization.
Abbreviations: dDDH, Digital DNA–DNA hybridization; LBA, Luria–Bertani agar; MA, MacConkey agar; NA, nutrient agar; R2A, Reasoner's 2A; TSA, tryptone soya agar.
The NCBI GenBank accession numbers for the 16S rRNA gene sequence and draft genome sequence of strains MAHUQ-68^T are MW488000 and JAMWYS000000000, respectively.
Three supplementary figures and three supplementary tables are available with the online Supplementary Material.

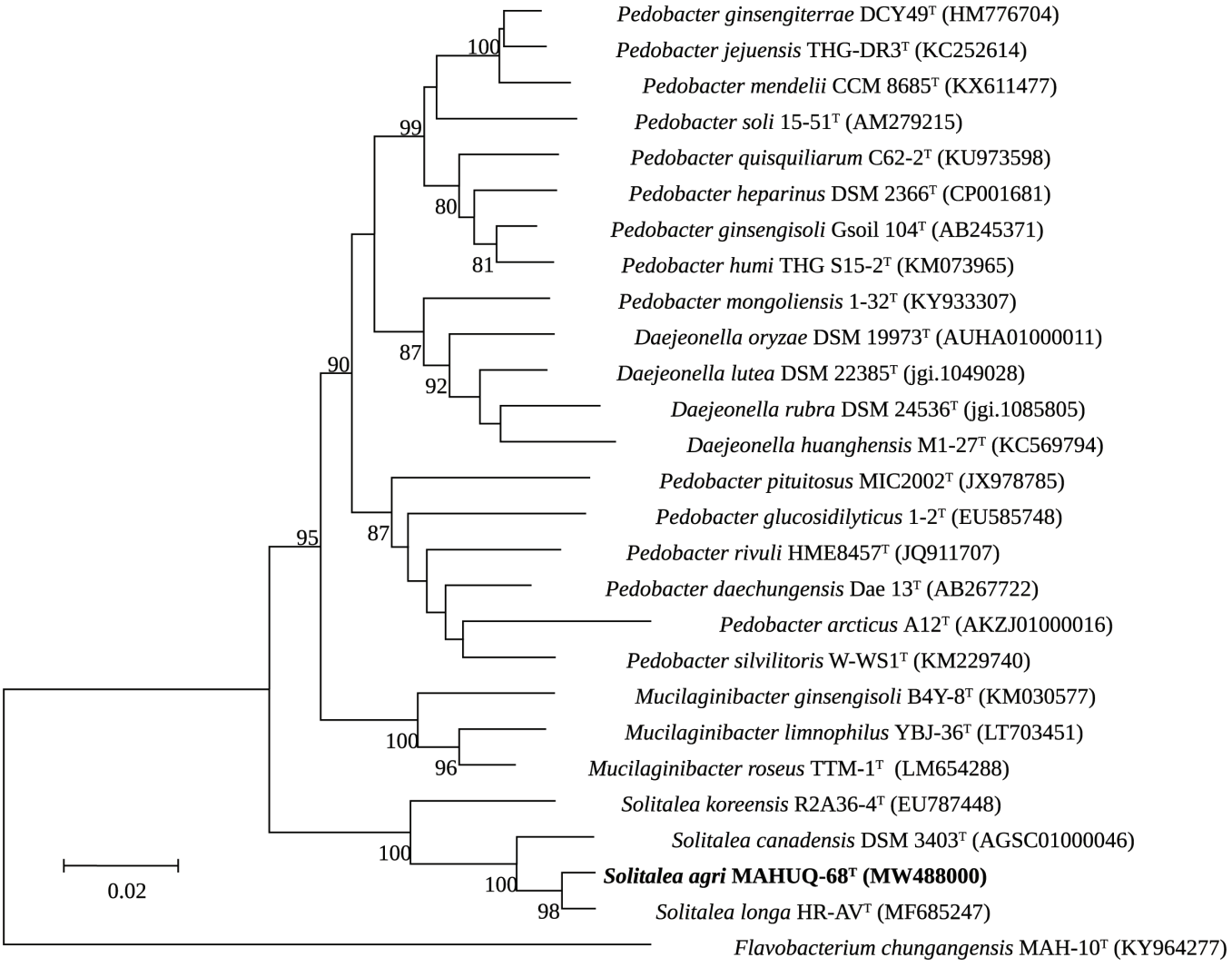

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of Solitalea *agri* MAHUQ-68^T and other related species. Bootstrap values more than 70% based on 1000 replications are shown at branching points. Flavobacterium *chungangensis* MAH-10^T was used as an outgroup. Scale bar, 0.02 substitutions per nucleotide position.

ISOLATION AND PRESERVATION

Strain MAHUQ-68^T was isolated from rhizospheric soil of a jujube tree located in Anseong, Republic of Korea (37° 00′ 31″ N 127° 22′ 33″ E) and subjected to polyphasic characterization. Briefly, 1 g rhizopheric soil sample was added into 9 ml distilled NaCl solution (0.85%, w/v), thoroughly stirred, and then diluted serially. Each dilution (100 µl) was spread on Reasoner's 2A (R2A) agar plates. Then, the plates were incubated for 3 days at 28 °C. Single colonies were purified by transferring onto new R2A agar plates. Isolation, purification and preservation of strain MAHUQ-68^T were done according to the previous description [6]. Strain MAHUQ-68^T has been deposited into the CGMCC and KACC. For the comparative study, the reference strains *S. longa* KACC 19411^T, *S. canadensis* JCM 21819^T and *S. koreensis* KACC 12953^T were included and tested using the same laboratory conditions as for strain MAHUQ-68^T.

PHYLOGENETIC ANALYSIS

Genomic DNA of strain MAHUQ-68^T was extracted using a genomic DNA extraction kit (Solgent). The 16S rRNA gene of strain MAHUQ-68^T was amplified by 27F forward and 1492R reverse primers [7]. The 16S rRNA gene was sequenced and assembled by Solgent Co. Ltd (Daejeon, Republic of Korea). Phylogenetically closest taxa were determined using the EzBioCloud server [8]. The 16S rRNA gene sequences of related taxa were collected from the EzBioCloud server and the NCBI GenBank database. All the collected 16S rRNA sequences along with strain MAHUQ-68^T were aligned using the ClustalX program [9]. The BioEdit program

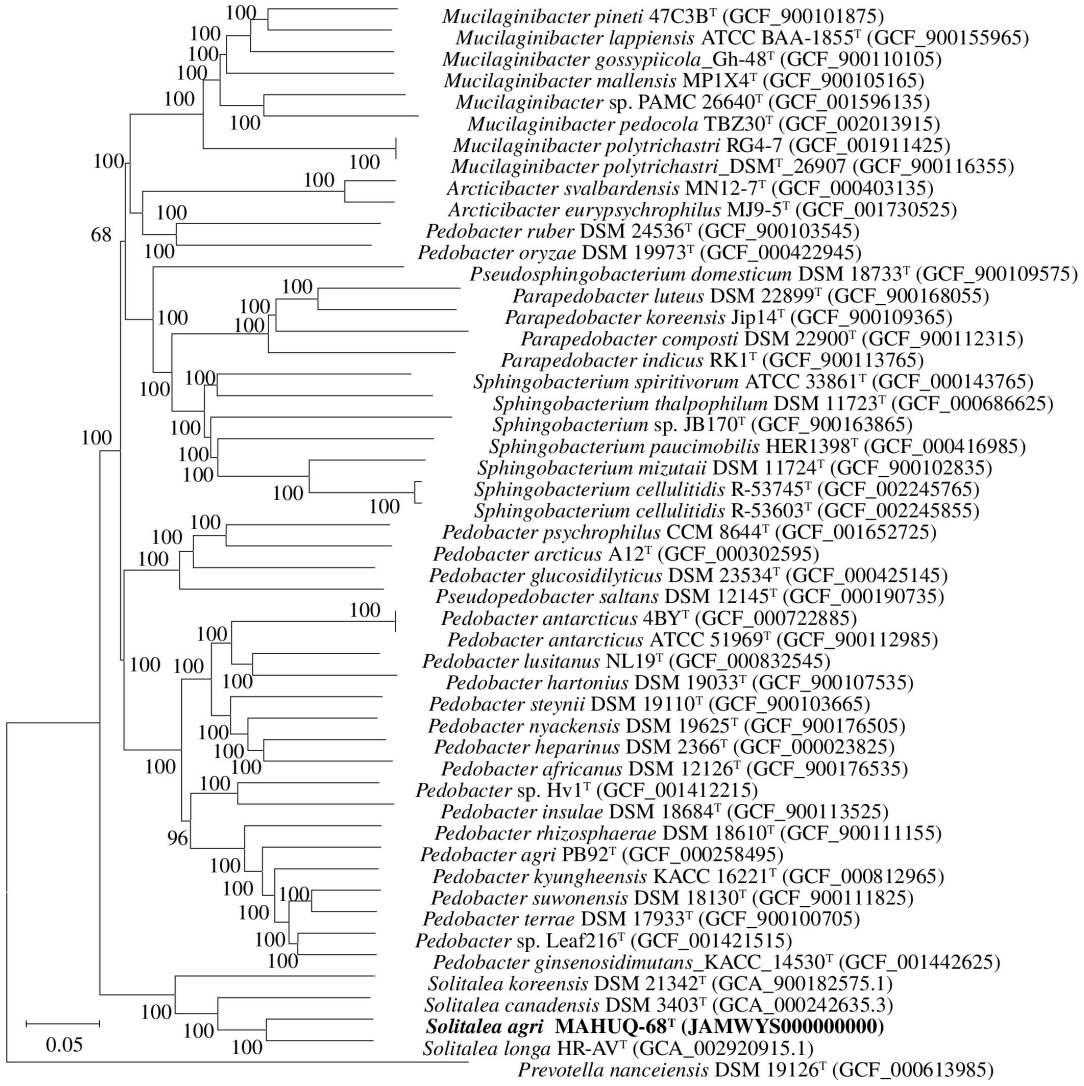

Fig. 2. Phylogenetic tree reconstructed from a comparative analysis of whole-genome sequences showing the relationships of strain MAHUQ-68^T with its closest related species. This tree was reconstructed *via* the Automated Multi-Locus Species Tree online web server and the MEGA 7 program using the aligned sequences of Automated Multi-Locus Species analysis. Bootstrap values (expressed as percentages of 1000 replications) greater than 60% are shown at the branch points. The bar represents 0.05 substitutions per nucleotide position.

was used to edit the gaps [10]. Neighbour-joining (NJ) and maximum-likelihood (ML) phylogenetic trees were reconstructed using MEGA 7.0 with bootstrap values based on 1000 replications [11].

The 16S rRNA gene sequence of novel strain MAHUQ-68^T was 1378 bp (NCBI GenBank/EMBL/DDBJ accession number MW488000). Based on the phylogenetic analysis using 16S rRNA gene and genome sequences, strain MAHUQ-68^T clustered together within the genus *Solitalea*. The closest members were *S. longa* HR-AV^T (98.8%), *S. canadensis* DSM 3403^T (96.9%) and *S. koreensis* R2A36-4^T (94.0%). Strain MAHUQ-68^T was clustered with *S. longa* HR-AV^T, *S. canadensis* DSM 3403^T and *S. koreensis* R2A36-4^T in both NJ and ML phylogenetic trees (Figs 1 and S1 available with the online version of this article) and formed a monophyletic clade with *S. longa* HR-AV^T. These phylogenetic positions of strain MAHUQ-68^T strongly support the classification of strain MAHUQ-68^T as a novel member of the genus *Solitalea*.

GENOME ANALYSES

For genome sequencing of strain MAHUQ-68^T, extraction of genomic DNA was performed using a genomic DNA extraction kit (Qiagen). Whole-genome sequencing of strain MAHUQ-68^T was carried out at the World Data Centre for Microorgannisms (Beijing, PR China) using the Illumina HiSeq platform. The whole genome sequences were assembled by the SOAPdenovo version

Table 1. Phenotypic differential characteristics of strain MAHUQ-68^T that differentiates with phylogenetically related type species of the genus *Solitalea*

Strains: 1, MAHUQ-68^T; 2, *Solitalea longa* KACC 19411^T; 3, *Solitalea canadensis* JCM 21819^T; 4, *Solitalea koreensis* KACC 12953^T. All data were obtained in this study, except [a] that was taken from Lee and Jeon [4]. All strains are motile and rod shaped. All strains are positive for 4-nitrophenyl-*β*-D-galactopyranoside, alkaline phosphatase, acid phosphatase, leucine arylamidase, valine arylamidase, cystine arylamidase, naphthol-AS-BI-phosphohydrolase, *N*-acetyl-*β*-glucosaminidase, α-glucosidase, α-galactosidase, *β*-glucosidase, *β*-galactosidase, hydrolysis of aesculin, assimilation of glucose, mannose and malic acid. All strains are negative for indole production, *β*-glucuronidase, hydrolysis of urea, Tween 20, and assimilation of capric acid and phenylacetic acid. +, Positive; w+, weakly positive; –, negative.

Characteristics	**1**	**2**	**3**	**4**
Isolation source	Rhizospheric soil	River	Soil	Greenhouse soil
Cell size (μm)	0.3–0.6×2.5–11.5	0.4–0.5×2.2–12.0[a]	0.3×3.0–60.0[a]	0.5–0.6×1.3–30.0[a]
Colony colour	Cream pink	Yellow	Light yellow	Light yellow
Aerobic/facultative anaerobic	Aerobic	Aerobic	Facultative anaerobic	Aerobic
Catalase	+	–	+	+
Oxidase	w	+	+	+
Reduction of nitrate (API 20 NE)	+	–	–	–
Fermentation of glucose	w	–	+	+
Arginine dihydrolase	–	–	+	+
Growth temperature (°C)	10–40	5–30[a]	10–40[a]	10–35[a]
Growth pH	6.0–9.0	7.0–8.0[a]	5.0–10.0[a]	5.0–8.0[a]
NaCl tolerance (%)	0–1.5	0-0.5[a]	<2[a]	0–1.0[a]
Hydrolysis of:				
Casein	+	–	+	–
Starch	w	–	+	–
L-Tyrosine	+	–	+	–
Tween 80	–	–	+	–
Enzyme activity (API ZYM):				
Esterase (C4)	w	+	+	+
Esterase lipase (C8)	w	+	+	+
Lipase (C14),	w	+	+	–
Trypsin	+	+	+	–
α-Chymotrypsin	+	+	+	–
α-Mannosidase	w	+	+	+
α-Fucosidase	–	+	+	+
Assimilation of (API 20 NE):				
L-Arabinose	+	–	–	+
N-Acetyl-glucosamine	+	+	–	+
Maltose	+	+	+	–
Mannitol	–	–	–	+
Gluconate	+	–	–	–
Adipic acid	–	–	–	+
Triosodium citrate	–	–	–	+

Table 2. Fatty acid profiles of strain MAHUQ-68^T and reference strains of the genus *Solitalea*

Strains: 1, MAHUQ-68^T; 2, *Solitalea longa* KACC 19411^T; 3, *Solitalea canadensis* JCM 21819^T;4, *Solitalea koreensis* KACC 12953^T. All data were obtained from this work. TR, Trace (less than 1.0%); ND, not detected.

Fatty acid	1	2	3	4
iso-$C_{13.0}$	1.7	TR	1.3	2.1
$C_{14:0}$	1.0	ND	ND	ND
iso-$C_{14.0}$	2.4	2.2	3.1	TR
$C_{15:0}$	ND	1.3	5.5	2.9
iso-$C_{15:0}$	32.2	30.1	38.3	28.0
anteiso-$C_{15:0}$	7.9	10.6	7.1	3.4
iso-$C_{15:0}$ 3OH	2.7	4.1	3.7	4.0
$C_{15:0}$ 2OH	TR	1.1	TR	TR
$C_{15:1}$ *ω6c*	2.7	TR	4.0	4.3
iso-$C_{16:0}$	2.5	2.1	3.4	1.5
$C_{16:0}$	4.3	2.1	3.7	3.0
iso-$C_{16:0}$ 3OH	TR	1.3	TR	1.1
$C_{16:0}$ 3OH	1.5	2.5	TR	TR
iso-$C_{16:1}$ h	1.4	TR	1.2	TR
$C_{16:1}$ *ω5c*	5.5	8.6	1.1	1.9
iso-$C_{17:0}$	TR	TR	TR	1.2
$C_{17:1}$ *ω6c*	2.2	TR	1.7	TR
iso-$C_{17:0}$ 3OH	8.0	9.2	5.2	13.1
$C_{19:0}$	ND	1.2	ND	TR
Summed feature 1*	TR	TR	TR	1.4
Summed feature 3†	16.5	13.8	10.3	17.0
Summed feature 4‡	1.6	1.4	1.9	1.3
Summed feature 9§	1.9	1.0	2.9	6.7

*Summed feature 1 contained $C_{13:0}$ 3-OH and/or iso-$C_{15:1}$ H.
†Summed feature 3 contained $C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*.
‡Summed feature 4 contained iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B.
§Summed feature 9 contained $C_{17:1}$ *ω9c* and/or $C_{16:0}$ 10-methyl.

3.10.1 *de novo* assembler. The whole-genome sequence of strain MAHUQ-68^T was annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP-6.1) [12] and the Rapid Annotation using Subsystem Technology server (RAST; https://rast.nmpdr.org) [13]. The genome sequences of reference strains were collected from the NCBI database. The genomic DNA G+C content of strain MAHUQ-68^T and three reference strains used in this study were measured based on their whole-genome sequences. The multilocus sequence typing (MLST) phylogenetic tree was also reconstructed using the whole-genome sequences based on multi-locus sequence analysis (https://automlst.ziemertlab.com/analyze) [14]. Genome-based relatedness values between MAHUQ-68^T and its three phylogenetically closest neighbours were calculated based on ANI (average nucleotide identity) *in silico* by the OrthoANIu algorithm (www.ezbiocloud.net/tools/ani) [15]. Digital DNA–DNA hybridization (dDDH) was calculated *in silico* by the Genome-to-Genome Distance Calculator using the BLAST method [16].

The whole-genome shotgun sequence of strain MAHUQ-68^T has been deposited at NCBI GenBank under the accession JAMWYS000000000. The genome size of strain MAHUQ-68^T is 4250173 bp with 68 contigs and the N50 value is 247993 bp. The genomic analysis revealed the presence of 3570 protein-coding genes including 54 tRNA and five rRNA genes (Table S1). The genomic DNA G+C content of strain MAHUQ-68^T is 38.0 mol%, which is within the range of *Solitalea* species [1, 4]. On

the other hand, the closest type strain, *S. longa* HR-AV^T, has a genome of 4517600 bp long with 36 contigs. The N50 value of the genome of *S. longa* HR-AV^T is 345499 bp and its genomic DNA G+C content is 38.4mol%. The genomic analysis revealed that *S. longa* HR-AV^T contains 3782 protein-coding genes including 56 tRNA and six rRNA genes. The MLST phylogenomic tree based on multi-locus sequence analysis showed that strain MAHUQ-68^T is clustered with the members of genus *Solitalea* and formed a monophyletic clade with *S. longa* HR-AV^T (Fig. 2). The RAST analysis of the genome of strain MAHUQ-68^T revealed the presence of 253 subsystems, 111 genes for respiration, 17 genes for stress responses, 38 genes for membrane transport, 37 genes for virulence, disease and defence, four genes for cell division and cell cycle, 62 genes for DNA metabolism, 206 genes involved with the metabolism of amino acids and derivatives, and 115 genes for carbohydrate metabolism. Strain MAHUQ-68^T does not have gene clusters for flagellar motility and chemotaxis (Table S2). The absence of genes for flagellar motility and the absence of flagella (Fig. S2) showed that the phenotypic and genomic results are consistent with each other. The RAST analysis of the genome of the closest strain *S. longa* HR-AV^T revealed the presence of 255 subsystems, 112 genes for respiration, 15 genes for stress responses, 40 genes for membrane transport, 40 genes for virulence, disease and defence, four genes for cell division and cell cycle, 64 genes for DNA metabolism, 210 genes involved with the metabolism of amino acids and derivatives, and 116 genes for carbohydrate metabolism (Table S2). The ANI values between strain MAHUQ-68^T and phylogenetically close neighbours *S. longa* HR-AV^T, *S. canadensis* DSM 3403^T and *S. koreensis* R2A36-4^T were 81.4, 74.1 and 72.0%, respectively (Table S3). The dDDH values between strain MAHUQ-68^T and *S. longa* HR-AV^T, *S. canadensis* DSM 3403^T and *S. koreensis* R2A36-4^T were 24.3, 19.8 and 20.6%, respectively (Table S3). The ANI values and dDDH values were much lower values than the species threshold of 95–96 and 70%, respectively, recommended for species delineation [16, 17]. These ANI and dDDH values clearly show that strain MAHUQ-68^T represents a novel member within the genus *Solitalea*.

PHENOTYPIC AND CHEMOTAXONOMIC CHARACTERIZATION

The cell morphology of strain MAHUQ-68^T was observed using transmission electron microscopy after growing on R2A agar plate for 2 days at 28 °C. The colony morphology of MAHUQ-68^T was investigated using a microscope (Olympus). Gram-staining reaction was conducted as described previously [18]. The cell motility test was performed using both sulphide indole motility medium (Oxoid) and microscopy. Catalase and oxidase activities of strain MAHUQ-68^T were checked using 3% (v/v) H_2O_2 and 1% (w/v) tetramethyl-*p*-phenylenediamine, respectively, according to the previous study [6]. Growth at various temperatures (4–45 °C) on R2A agar plates was investigated for 7 days. Growth was observed on different bacteriological media such as Luria–Bertani agar (LBA; Oxoid), R2A agar (MB cell), nutrient agar (NA; Oxoid),), tryptone soya agar (TSA; Oxoid) and MacConkey agar (MA; Oxoid). DNase activity of strain MAHUQ-68^T was checked using DNase agar (Oxoid). Salt tolerance was examined in R2A broth supplemented with different concentrations of NaCl (0–5%, at 0.5% intervals). The range of growth pH was examined in R2A broth (pH 4.0–11.0, at 0.5 pH unit intervals) at 28 °C for 7 days. Hydrolysis of L-tyrosine, starch, casein, gelatin, Tweens 80 and Tween 20 was investigated according to the description of Smibert and Krieg [19]. Anaerobic growth of strain MAHUQ-68^T was checked for 14 days on R2A agar at 28 °C using AnaeroGen kit (Thermo Scientific). Synthesis of flexirubin-type pigments by strain MAHUQ-68^T was monitored by the previous description [20]. Other physiological and biochemical tests were performed using API 20NE and API ZYM kits (bioMérieux) following the manufacturer's instructions.

The cells of strain MAHUQ-68^T were observed to be Gram-stain-negative, strictly aerobic, non-flagellated and rod-shaped (0.3–0.6 µm wide and 2.5–11.5 µm long) (Fig. S2). Positive for both catalase and oxidase activities. Strain MAHUQ-68^T was able to hydrolyse tyrosine, aesculin, casein and starch, but not gelatin, DNA, urea, Tweens 80 and Tween 20. The colonies were cream pink coloured, spherical and 1.5–3.5 mm in diameter when grown on R2A agar medium for 2 days at 28 °C. Colonies grew at 10–40 °C (optimum, 28 °C), pH 6.0–9.0 (optimum pH, 7.0) and in the presence of 0–1.5% NaCl (optimum 0–0.5%). Strain MAHUQ-68^T was positive for the following enzyme activities: acid phosphatase, alkaline phosphatase, cysteine arylamidase, leucine arylamidase, trypsin, valine arylamidase, naphthol-AS-BI-phosphohydrolase, *β*-glucosidase, *N*-acetyl-*β*-glucosaminidase, *α*-galactosidase, *β*-galactosidase, *α*-chymotrypsin, esterase (C4), α-mannosidase, esterase lipase (C8), α-glucosidase and lipase (C14). Morphological and biochemical investigations showed that strain MAHUQ-68^T shared several traits in common with the closest relatives. However, there are some clear differences in the morphological, physiological and biochemical characteristics between strain MAHUQ-68^T and closely related species that differentiate the strain MAHUQ-68^T from closely related type strains (Table 1). Strain MAHUQ-68^T can be easily differentiated from phylogenetically closest type species *S. longa* HR-AV^T by catalase activity, reduction ability of nitrate, hydrolysis ability of casein and L-tyrosine, assimilation of L-arabinose and gluconate, different growth conditions, *etc*. The differential physiological and biochemical characteristics of strain MAHUQ-68^T are given in Table 1 along with those of its three closest reference strains.

For the identification of cellular fatty acids, cells of strain MAHUQ-68^T and three reference strains were harvested from identical culture conditions (R2A agar medium, 2 days incubation at 28 °C). Fatty acid methyl esters were extracted using the MIDI protocol and analysed using a gas chromatograph [21]. The isoprenoid quinones of strain MAHUQ-68^T were extracted from freeze-dried cells using the protocol of Minnikin *et al.* [22]. The extracted quinones were analysed using HPLC according to the previous description [23]. Polar lipids of strain MAHUQ-68^T were extracted from dry cells as described by Minnikin *et al.* [22] with modifications. Briefly, 80 mg freeze-dried cells were suspended in 1.5 ml of 0.3% saline in a screw-capped tube. Then, 10 ml methanol

was added and heated at 100 °C for 5 min. After cooling, 5 ml chloroform and 3 ml saline were added and kept in a shaker for 3 h. Then, the supernatant was collected through centrifugation at 5000 r.p.m. for 10 min. Chloroform and saline (5 ml each) were added to the supernatant. Finally, the down layer (chloroform layer) was collected and concentrated to dryness. The extracted polar lipids were dissolved in 100 µl chloroform–methanol (2:1, v/v). The samples (10 µl) were spotted on the corner of 2D-TLC plates (10×10 cm) using TLC Kiesel gel 60F254 (Merck) and developed in the first direction by using chloroform–methanol–water (65:25:4, by v/v/v) while in the second direction developed by chloroform–acetic acid–methanol–water (80:15:12:4, by v/v/v) as solvent systems. The total polar lipids, aminolipids, glycolipids and phospholipids were detected by staining the plates with 5% molybdophosphoric acid, 0.2% ninhydrin, 15% α-naphthol (dissolved in 95% ethanol) and molybdenum blue, respectively [24].

The major cellular fatty acids of strain MAHUQ-68^T were iso-$C_{15:0}$ (32.2%) and summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*, 16.5%), similar to previous results for the genus *Solitalea*. Strain MAHUQ-68^T also contained a considerable amount of anteiso-$C_{15:0}$ (7.9%), iso-$C_{17:0}$ 3OH (8.0%) and $C_{16:0}$ (4.3%), which is also similar to the members of the genus *Solitalea*. However, quantitative differences in the major and minor fatty acids differentiate strain MAHUQ-68^T from other closely related species of the genus *Solitalea* (Table 2). The main respiratory quinone of strain MAHUQ-68^T was menaquinone-7 (MK-7) which is also one of the characteristics of the genus *Solitalea* [1, 4]. The polar lipids identified in strain MAHUQ-68^T were phosphatidylethanolamine, an unidentified aminolipid and four unidentified lipids (Fig. S3). The predominant polar lipids of strain MAHUQ-68^T were similar to those of the most closely related type strains [4].

In summary, as indicated by the phylogenetic tree results, strain MAHUQ-68^T belongs to the genus *Solitalea*. In addition, the characteristics of strain MAHUQ-68^T are consistent with descriptions of the genus *Solitalea* with regard to morphological, biochemical and chemotaxonomic properties. However, strain MAHUQ-68^T can be distinguished from the closely related type strains not only by physiological and biochemical characteristics but also by low dDDH values and ANI values. The results of this polyphasic comparison between strain MAHUQ-68^T and its close phylogenetic neighbours indicated that strain MAHUQ-68^T should be assigned to the genus *Solitalea* as a novel species, for which the name *Solitalea agri* sp. nov. is proposed.

DESCRIPTION OF *SOLITALEA AGRI* SP. NOV.

Solitalea agri (L. gen. n. *agri*, of a field).

Cells (0.3–0.6 µm wide and 2.5–11.5 µm long) are rod-shaped, strictly aerobic, Gram-stain-negative and motile by gliding without flagellum. Colonies (1.5–3.5 mm) are cream pink coloured, smooth and spherical on R2A agar plate after 2 days at 28 °C. Colonies grow well on R2A agar and NA, no growth is observed on TSA, LB or MacConkey agar. Colonies grow at 10–40 °C (optimum, 28 °C) and pH 6.0–9.0 (optimum pH, 7.0). Cells grow optimally in the presence of 0–0.5% NaCl but tolerate up to 1.5% (w/v) of NaCl. Positive for catalase and oxidase tests. Nitrate is reduced to nitrite. Flexirubin-type pigments are absent and negative for indole production. Glucose is weakly fermented. Cells were able to hydrolyse casein, starch, L-tyrosine and aesculin but not gelatin, L-arginine, DNA, Tween 20, Tween 80 or urea. The type strain MAHUQ-68^T shows the following enzyme activities: positive for acid phosphatase, alkaline phosphatase, cysteine arylamidase, leucine arylamidase, trypsin, valine arylamidase, naphthol-AS-BI-phosphohydrolase, *β*-glucosidase, N-acetyl-*β*-glucosaminidase, *α*-galactosidase, *β*-galactosidase; *α*-chymotrypsin and *α*-glucosidase; weakly positive for esterase (C4), esterase lipase (C8), *α*-mannosidase and lipase (C14); and negative for *β*-glucuronidase and *α*-fucosidase (API ZYM). Glucose, mannose, maltose, L-arabinose, gluconate, *N*-acetyl-glucosamine and malic acid are assimilated, but, the following compounds are not assimilated: mannitol, adipic acid, capric acid, triosodium citrate and phenylacetic acid (API 20NE). The major cellular fatty acids are iso-$C_{15:0}$ and summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*). The polar lipids comprise phosphatidylethanolamine, an unidentified aminolipid and four unidentified lipids. The main respiratory quinone is menaquinone-7. The genomic DNA G+C content of the type strain is 38.0 mol%.

The type strain, MAHUQ-68^T (=KACC 22249^T=CGMCC 1.19062^T), was isolated from rhizospheric soil of jujube tree, located in Naeri, Anseong, Republic of Korea.

Funding Information
No external fund was received for this study.

Acknowledgements
The authors give special thanks to the CGM 10K project for analyzing the draft genome sequence of strain MAHUQ-68^T (GCM60023251).

Author contributions
M.A.H. and S.A. conceived the study, designed and performed the experiments, analysed the results and wrote the article. S.Y.L, B.K.M., C.C. and S.K.M analysed some data and checked the manuscript. All authors thoroughly revised the manuscript and approved its submission.

Conflicts of interest
The authors declare that there are no conflicts of interest.

References

1. Weon H-Y, Kim B-Y, Lee C-M, Hong S-B, Jeon Y-A, *et al*. *Solitalea koreensis* gen. nov., sp. nov. and the reclassification of [*Flexibacter*] *canadensis* as *Solitalea canadensis* comb. nov. *Int J Syst Evol Microbiol* 2009;59:1969–1975.
2. Steyn PL, Segers P, Vancanneyt M, Sandra P, Kersters K, *et al*. Classification of heparinolytic bacteria into a new genus, *Pedobacter*, comprising four species: *Pedobacter heparinus* comb. nov., *Pedobacter piscium* comb. nov., *Pedobacter africanus* sp. nov. and *Pedobacter saltans* sp. nov. proposal of the family *Sphingobacteriaceae* fam. nov. *Int J Syst Bacteriol* 1998;48 Pt 1:165–177.
3. Krieg NR, Ludwig W, Euzeby J, Phylum WWB, *et al*. Phylum XIV. Bacteroidetes phyl. nov. In: Krieg NR, Staley JT, Brown DR, Hedlund BP and Paster BJ (eds). *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 4. New York: Springer; 2011. p. 25.
4. Lee Y, Jeon CO. *Solitalea longa* sp. nov., isolated from freshwater and emended description of the genus *Solitalea*. *Int J Syst Evol Microbiol* 2018;68:2826–2831.
5. Christensen P. *Flexibacter canadensis* sp. nov. *IntJ Syst Bacteriol* 1980;30:429–432.
6. Huq MA, Akter S, Siddiqi MZ, Balusamy SR, Natarajan S, *et al*. *Sphingobium tyrosinilyticum* sp. nov., a tyrosine hydrolyzing bacterium isolated from Korean radish garden. *Arch Microbiol* 2018;200:1143–1149.
7. Frank JA, Reich CI, Sharma S, Weisbaum JS, Wilson BA, *et al*. Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. *Appl Environ Microbiol* 2008;74:2461–2470.
8. Yoon S-H, Ha S-M, Kwon S, Lim J, Kim Y, *et al*. Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 2017;67:1613–1617.
9. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 1997;25:4876–4882.
10. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucl Acids Symp Ser* 1999;41:95–98.
11. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol Biol Evol* 2016;33:1870–1874.
12. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, *et al*. NCBI prokaryotic genome annotation pipeline. *Nucleic Acids Res* 2016;44:6614–6624.
13. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, *et al*. The RAST Server: rapid annotations using subsystems technology. *BMC Genomics* 2008;9:75.
14. Alanjary M, Steinke K, Ziemert N. AutoMLST: an automated web server for generating multi-locus species trees highlighting natural product potential. *Nucleic Acids Res* 2019;47:W276–W282.
15. Yoon S-H, Ha S-M, Lim J, Kwon S, Chun J. A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie van Leeuwenhoek* 2017;110:1281–1286.
16. Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 2013;14:60.
17. Richter M, Rosselló-Móra R. Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci U S A* 2009;106:19126–19131.
18. Akter S, Wang X, Lee S-Y, Rahman MM, Park J-H, *et al*. *Paenibacillus roseus* sp. nov., a ginsenoside-transforming bacterium isolated from forest soil. *Arch Microbiol* 2021;203:3997–4004.
19. Smibert RM, Krieg NR. Phenotypic characterization. In: Gerhardt P, Murray RGE, Wood WA and Krieg NR (eds). *Methods for General and Molecular Bacteriology*. Washington: American Society for Microbiology; 1994. pp. 607–654.
20. Akter S, N A M K, Lee S-Y, Moon S-K, Choi C, *et al*. *Ramlibacter pinisoli* sp. nov., a novel bacterial species isolated from pine garden soil. *Int J Syst Evol Microbiol* 2020;70:5841–5847.
21. Sasser M. Bacterial identification by gas chromatographic analysis of fatty acid methyl esters (GC-FAME). MIDI Tech Note 101. Newark, MIDI Inc; 1990
22. Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M, *et al*. An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* 1984;2:233–241.
23. Akter S, Park JH, Rahman MM, Huq MA. *Niastella soli* sp. nov., isolated from rhizospheric soil of a persimmon tree. *Int J Syst Evol Microbiol* 2021;71.
24. Akter S, Huq MA. *Sphingomonas chungangi* sp. nov., a bacterium isolated from garden soil sample. *Int J Syst Evol Microbiol* 2020;70:4151–4157.