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Short Communication

e-Xtra*

Genomic Analyses of Rose Crown Gall-Associated Bacteria Revealed Two New *Agrobacterium* Species: *Agrobacterium burrii* sp. nov. and *Agrobacterium shirazense* sp. nov.

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ABSTRACT

Agrobacterium tumefaciens species complex contains a set of diverse bacterial strains, most of which are well known for their pathogenicity on agricultural plants causing crown gall diseases. Members of *A. tumefaciens* species complex are classified into several taxonomically distinct lineages called "genomospecies" (13 genomospecies until early 2021). Recently, two genomospecies, G19 (strains Rnr^T, Rew, and Rnw) and G20 (strains OT33^T and R13) infecting *Rosa* sp. plants in Iran, were described based on biochemical and molecular-phylogenetic data. Whole genome sequence-based core-genome phylogeny followed by average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) calculations performed in this study suggested that genomospecies G19 and G20 could be described as two novel and standalone species. In the phylogenetic tree, these two new genomospecies were clustered separately from other genomospecies/species of *A. tumefaciens* species complex. Moreover, both ANI and dDDH indices between the G19/G20 strains and other *Rhizobiaceae* members are clearly below the accepted thresholds for prokaryotic species description. Hence, *Agrobacterium burrii* sp. nov. is proposed to encompass the G19 strains, with Rnr^T = CFBP 8705^T = DSM 112541^T as type strain. *Agrobacterium shirazense* sp. nov. is also proposed to include G20 strains, with OT33^T = CFBP 8901^T = DSM 112540^T as type strain.

Keywords: agrobacteria, genomospecies, Iran, *Rhizobiaceae*, Shiraz, tumorigenesis

Members of the bacterial family *Rhizobiaceae* are well known for their contribution to nitrogen fixation in plant rhizosphere (rhizobia) and their pathogenicity on a vast number of agricultural plants causing crown gall and hairy root diseases (agrobacteria) (de Lajudie et al. 2019; Puławska 2016). Until recently, classification of *Rhizobiaceae* has been a matter of conflict among scientists, which is largely resolved nowadays because of availability of whole genome sequence data. Initially, taxonomy of agrobacteria was designed on the basis of symptomatology of host plants (Allen and Holding 1974). During the 1970s, physiological and biochemical analyses suggested division of agrobacteria into three biovars (Kersters and De Ley 1984), which was then confirmed by chemotaxonomy, DNA-DNA hybridization, and 16S rDNA sequence analyses (Flores-Félix et al. 2020). Subsequently, biovar 1 was referred to as *A. tumefaciens* species complex and 13 genomic species (genomospecies; i.e., G1-G9, G13, G14, G19, and G20) were defined within the *A. tumefaciens* species complex based on DNA-DNA hybridization, AFLP scheme, and multilocus sequence analysis (Mafakheri et al. 2019; Mougel et al. 2002; Portier et al. 2006; Puławska and Kałużna 2011). Since the beginning of the genomics era, availability of complete genome sequence data led to a substantial improvement in the taxonomy of *Rhizobiaceae*. Seven of 13 genomospecies received formal species description: *A. fabacearum* (G1; Delamuta et al. 2020), *A. nepotum* (G14; Puławska et al. 2012), *A. pusense* (G2; Panday et al. 2011), *A. radiobacter* (G4; Lindstrom and Young 2011), *A. salinitolerans* (G9; Yan et al. 2017b), "*A. deltaense*" (G7; Yan et al. 2017a), and "*A. fabrum*" (G8; Lassalle et al. 2011), whereas six genomospecies remain without taxonomically valid species names.

During 2014 to 2019, dozens of agrobacterial strains were isolated from crown gall tissues of different annual and perennial plants: *Beta vulgaris* (sugar beet), *Ficus benjamina* (weeping fig), *Malus pumila* (apple), *Prunus persica* (peach), *Prunus persica* var. nucipersica (nectarine), *Rosa* spp. (rose), and *Vitis vinifera* (grapevine) in Iran (Mafakheri et al. 2017a, b, 2019). Preliminary characterizations such as pathogenicity test, specific PCRs, and multilocus sequence analysis, suggested that most of the strains belonged to either the already known species (i.e., *Allorhizobium vitis*, *A. larrymoorei*, and *A. rubi*) or the *A. tumefaciens* species complex (*A. fabacearum* [G1]; *A. radiobacter* [G4]; "*A. deltaense*" [G7]; and *A. nepotum* [G14]). However, three atypical phylogenetic clades were observed within the strains isolated in Iran, whereby two novel genomospecies within the *A. tumefaciens* species complex were proposed (Mafakheri et al. 2019). Genomospecies G19 included the strains Rew, Rnw, and Rnr^T; G20 included the strains R13 and OT33^T, whereas the phylogenetic status of the apple strain Ap1

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remained undetermined (Mafakheri et al. 2019). Additionally, in 2019, another atypical strain was isolated from Japanese spindle (*Euonymus japonicus* 'Green Rocket') in Iran (Mafakheri et al. 2021). The purpose of the present study was to clarify taxonomic status of the four atypical agrobacterial clades and provide species description for the G19 and G20 strains.

The four bacterial strains A.E1, Ap1, OT33^T, and Rnr^T were streaked onto nutrient agar (NA) medium and incubated at 27°C for 3 to 4 days. The strains were resuspended in sterile distilled water and stored at 4°C for further use while they were maintained in 15% glycerol at −70°C for long-term use (Mafakheri et al. 2019). Furthermore, a pure culture of the strains was deposited in the CIRM-CFBP (French Collection for Plant-Associated Bacteria) and DSMZ (German Collection of Microorganisms and Cell Cultures) culture collections with assigned accession numbers as follows: A.E1 = CFBP 8903; Ap1 = CFBP 8706; OT33^T = CFBP 8901^T = DSM 112540^T; and Rnr^T = CFBP 8705^T = DSM 112541^T.

Phenotypic features and biochemical characteristics (Table 1) of the strains were determined using the standard procedure described in the literature (Moore et al. 2001). Standard strains of several agrobacterial strains were used as control. The strains designated as G19 and G20 (Rnr^T and OT33^T, respectively) were able to grow on 2% NaCl but not in 4% NaCl, whereas strains of "*A. deltaense*" (YIC4121^T), "*A. fabrum*" (C58), *A. larrymoorei* (Ficamol), *A. pusense* (NRCPB10^T), *A. radiobacter* (ICMP 5856^T), *A. rosae* (A.E1), and *Rhizobium cellulosilyticum* (Ap1) grow on NA medium supplemented with 4% NaCl (Lassalle et al. 2011; Yan et al. 2017a). The strains Ap1 and A.E1 as well as *A. salinitolerans* (YIC 5082^T) grow on NA medium supplemented with 5% NaCl (Yan et al. 2017b). *A. salinitolerans* isolated from root nodules of *Sesbania cannabina* grew in a high-salt and alkaline environment (Yan et al. 2017b), whereas Ap1 and A.E1 were isolated from apple and Japanese spindle.

The strains Rnr^T and OT33^T, unlike Ap1 and A.E1, produced 3-ketolactose from lactose, whereas the two former strains varied from each other in the use of citrate in which OT33^T such as *A. arsenijevicii* (KFB 330^T) could use citrate but Rnr^T and other *Agrobacterium* species such as "*A. bohemicum*" (R90^T), "*A. deltaense*" (YIC4121^T), "*A. fabrum*" (C58), *A. larrymoorei* (Ficamol), *A. nepotum* (39/7^T), *A. pusense* (NRCPB10^T), *A. radiobacter* (ICMP 5856^T), and *A. skierniewicense* (Ch11^T) could not do so (Kuzmanović et al. 2015). Strains OT33^T and Rnr^T were clearly different from *A. radiobacter* (ICMP 5785^T) based on use of D-mannitol and L-rhamnose, whereas the former strains unlike *A. radiobacter* (ICMP 5785^T) were able to use D-mannitol and L-rhamnose (Panday et al. 2011). The four strains investigated in this study varied from each other in the use of adonitol, D-maltose, D-sorbitol, dulcitol, and xylose, as detailed in Table 1. The strain OT33^T was able to use all these carbon sources, whereas the Rnr^T strain was unable to use dulcitol and xylose.

Genomic DNA extraction was performed using the procedure described previously (Mafakheri et al. 2021). DNA libraries were obtained with Nextera XT DNA Library Prep Kit (Illumina). Paired-end sequencing (2 × 150 bp) was performed on an Illumina HiSeq X platform generating 2,407,614 (A.E1); 2,188,977 (Ap1); 4,856,053 (OT33^T); and 2,120,000 (Rnr^T) paired reads. Reads were demultiplexed using BaseSpace (Illumina). Pair reads' quality filtering and trimming were performed with the bbduk program (Bushnell 2014). Adaptor trimming was performed using Trimmomatic (Galaxy version 0.38.1) (Bolger et al. 2014) implemented on the Galaxy web server (Afgan et al. 2018). The read quality was assessed with FastQC (Galaxy version 0.72+galaxy1; https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). De novo sequence assembly was performed using SPAdes genome assembler (Bankevich et al. 2012) (Galaxy version 3.12.0+galaxy1). Genome annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline with default settings (Borodovsky and Lomsadze 2014). Genome length (bp), G + C content (%), total number of protein-coding genes, RNA genes, and pseudogenes were determined for all genomes as shown in Supplementary Table S1. Genome size of the strains varied from 5,514 to 6,423 kbp in OT33^T and Ap1, respectively (Supplementary Table S1). Further, G + C% content of the strains was between 56.5% in A.E1 and 59.9% in OT33^T.

To determine the precise phylogenetic position of the strains sequenced in this study, whole genome sequences of representative *Rhizobiaceae* species were retrieved from the NCBI GenBank database (Supplementary Table S3) and included in the phylogenetic analyses. The dataset included representatives of *Rhizobiaceae* genera and *Agrobacterium* species and genomospecies described so far. In addition, we included *Agrobacterium* spp. strains closely related to genomospecies G19 and G20. These additional strains were selected by performing BLASTn searches (https://blast.ncbi.nlm.nih.gov/Blast.cgi) for *recA* gene sequences of G19 and G20 strains against the NCBI WGS and NR/NT databases (last accessed in February 2021). For core-genome phylogenetic analysis, computations of homologous gene clusters were performed by bidirectional best-hit (BDBH), Clusters of Orthologous Groups-triangles (COGtriangles), and OrthoMCL (Markov Clustering of orthologs, OMCL) algorithms with a stringent 90% coverage cut-off for BLASTP alignments (-C 90) using the script get_homologues.pl implemented into GET_HOMOLOGUES software package version 11042019 (Contreras-Moreira and Vinuesa 2013). A consensus core-genome was computed as the intersection of the clusters computed by the BDBH,

TABLE 1. Phenotypic characteristics and biochemical features of the four *Rhizobiaceae* strains investigated in this study along with a representative set of *Agrobacterium* spp. used as control[a]

Characteristics	*Agrobacterium burrii* Rnr^T	*A. shirazense* OT33^T	*Rhizobium cellulosilyticum* Ap1	*A. rosae* AE.1	*A. radiobacter* ICMP 5856^T	"*A. fabrum*" C58	*A. larrymoorei* Ficamol	"*A. bohemicum*" R90^T	"*A. deltaense*" YIC4121^T	*A. arsenijevicii* KFB 330^T	*A. nepotum* 39/7^T	*A. pusense* NRCPB10^T	*A. skierniewicense* Ch11^T	*A. rubi* TR3^T
Growth in 2% NaCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth in 4% NaCl	–	–	+	+	+	+	+	–	+	–	W	+	W	–
Growth in 5% NaCl	–	–	+	+	–	–	–	–	–	–	–	–	–	–
Growth in 6% NaCl	–	–	–	–	–	–	–							
3-Ketolactose	+	+	–	–	+	+	+	–	ND	+	+	+	–	–
Citrate use	–	+	+	–	–	–	–	–	–	+	–	–	–	–
Use of														
Adonitol	+	+	–	–	+	+	+	+	ND	ND	+	+	+	ND
D-cellobiose	+	+	+	+	+	+	+	ND	ND	+	+	+	–	ND
D-fructose	+	+	+	+	+	+	+	ND	ND	+	+	+	+	ND
D-maltose	+	+	–	+	+	–	+	–	ND	+	+	+	+	ND
D-mannitol	+	+	+	+	–	+	+	+	ND	+	+	+	+	–
D-raffinose	+	+	+	+	+	+	–	ND	ND	+	+	–	+	+
D-sorbitol	+	+	–	+	+	+	–	+	+	ND	+	+	–	ND
Dulcitol	–	+	+	+	+	–	–	ND	ND	ND	ND	ND	+	ND
Inositol	+	+	+	+	+	+	+	ND	ND	+	+	+	–	–
L-arabinose	+	+	+	+	+	+	+	ND	ND	ND	+	–	+	–
L-rhamnose	+	+	+	+	–	–	+	+	ND	ND	+	–	+	ND
Xylose	–	+	+	+	+	+	+	ND	ND	ND	–	ND	+	ND

[a] ND, not determined; and W, weak.

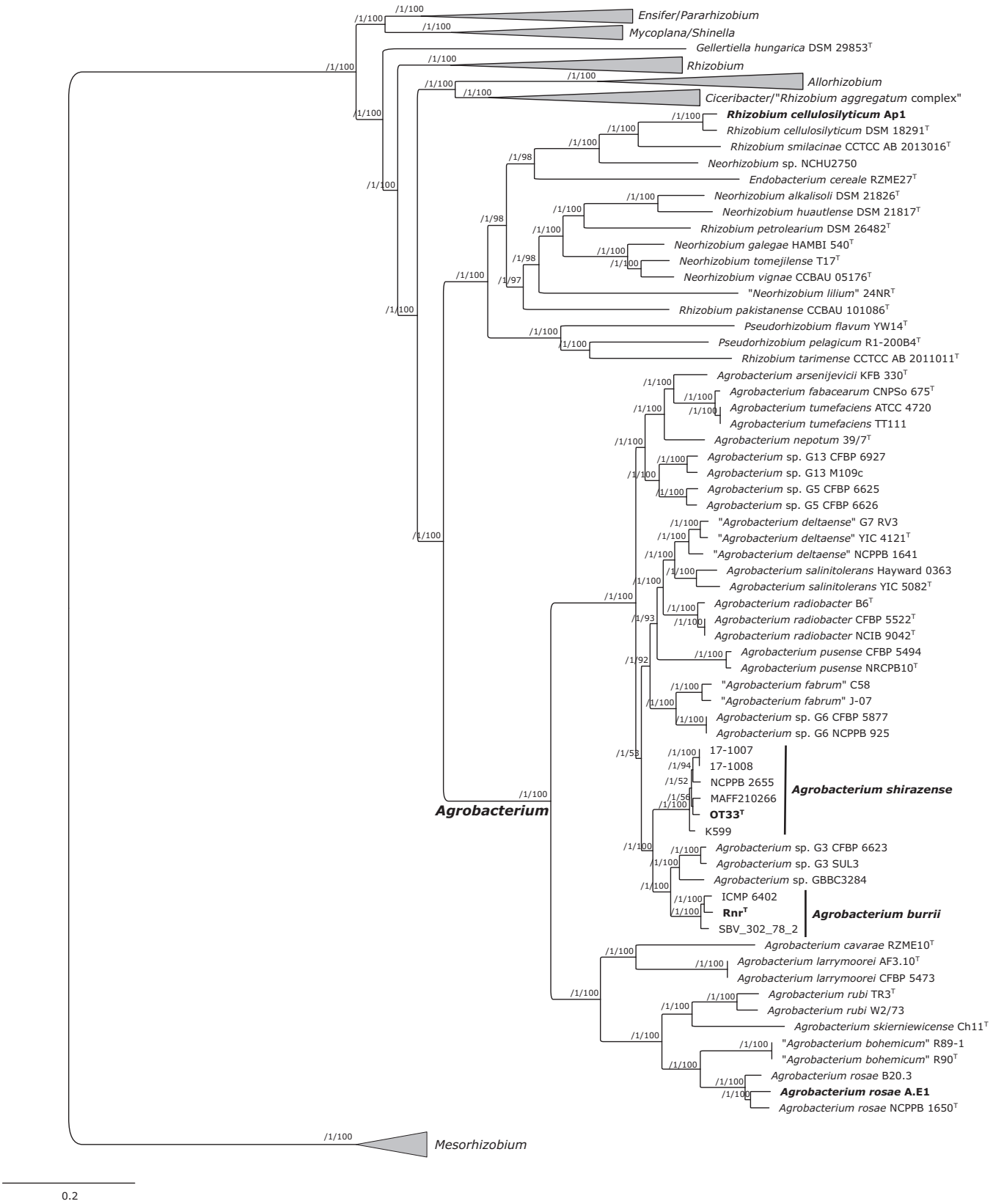

Fig. 1. Core genome-based phylogenetic tree of the four strains sequenced in this study along with an entire set of *Rhizobiaceae* species (Supplementary Table S3). The tree was estimated with IQ-TREE from the concatenated alignment of 341 top-ranked genes selected using GET_PHYLOMARKERS software. The numbers on the nodes indicate the approximate Bayesian posterior probabilities support values (first value) and ultra-fast bootstrap values (second value), as implemented in IQ-TREE (Nguyen et al. 2015). The tree was rooted using the sequences of representatives of the genus *Mesorhizobium* as the outgroup. The scale bar represents the number of expected substitutions per site under the best-fitting GTR+F+ASC+R7 model. The strains Rnr^T and OT33^T represent new species *Agrobacterium burrii* sp. nov. and *A. shirazense* sp. nov, respectively, described in this study. The strain Ap1 isolated from apple crown gall was identified as *Rhizobium cellulosilyticum*, whereas the strain A.E1 isolated from Japanese spindle was identified as *Agrobacterium rosae*.

COG-triangles, and OMCL algorithms by employing script compare_clusters.pl (-t 83, number of genomes). The resulting core-genome clusters were used as an input for phylogenomic analysis using the pipeline for DNA-based phylogenies (-R 1 -t DNA) of GET_PHYLOMARKERS software package version 2.2.8_18Nov2018 (Vinuesa et al. 2018). Based on the resulting core-genome phylogenetic tree (Fig. 1), average nucleotide identity (ANI) was calculated among the agrobacterial genome sequences representing members of different species and genomospecies. The ANI was estimated using both one-versus-one and all-versus-all strategies via different algorithms: ANIb in JSpeciesWS (Richter et al. 2016); ANI calculator (Rodriguez and Konstantinidis 2016), and OrthoANIu (Yoon et al. 2017). Additionally, Genome-to-Genome Distance Calculator (version 2.1) online service was used to calculate digital DNA-DNA hybridization (dDDH) value, which infers the genome-to-genome distances between pairs of genomes based on the Genome Blast Distance Phylogeny (Meier-Kolthoff et al. 2013). A combination of ANI and dDDH indices was used to assign a standalone species taxonomic status to a given taxon. When both ANI and dDDH values were below the accepted threshold for prokaryotic species description (i.e., ≤95% and ≤70% for ANI and dDDH, respectively), the corresponding strain was considered a potential novel species (Kim et al. 2014).

Core-genome-based phylogenetic analyses showed that the strain Ap1 was clustered with type strain of *R. cellulosilyticum* as its closest relative. These two strains shared 96.3% ANI and 71.7% dDDH values with each other. Taken together, our results indicated that strain Ap1 belong to the species *R. cellulosilyticum*, although ANI and dDDH values were just slightly above the thresholds for species delineation. On the other hand, the strain A.E1 was identified as *A. rosae* based on its high sequence similarity to the type strain of the species NCPPB 1650^T (ANI = 96.9%, dDDH = 72.4%; Supplementary Table S2). The strain A.E1 has previously been phenotypically investigated and was shown to be pathogenic on sunflower and tomato test plants under greenhouse conditions (Mafakheri et al. 2021).

In the core-genome phylogenetic tree, the strain OT33^T clustered among several taxonomically undetermined *Agrobacterium* sp. strains (i.e., NCPPB 2655, MAFF 210266, 17-1007, 17-1008, and K599), whose genome sequences were retrieved from GenBank and included in the analysis (Fig. 1; Supplementary Table S3). ANI values between the strain OT33^T and its closest neighbors in the same clade were varied between 97.1 and 97.8%, as detailed in Table 2. dDDH values among the members of this clade were between 82.4 and 84.4%. Hence, all strains were considered members of the same species, for which no formal description and protolog are available in the literature. Furthermore, both ANI and dDDH values between the strains representing new species (OT33^T, NCPPB 2655, MAFF 210266, 17-1007, 17-1008, and K599) and the representative strains throughout *Rhizobiaceae* were less than the accepted threshold for prokaryotic species definition (Supplementary Table S2). The strains NCPPB 2655, MAFF 210266, K599, 17-1007, and 17-1008 were isolated from different dicotyledonous plants (i.e., cucumber and *Lantana* sp.) but their pathogenicity on their host of isolation remains undetermined. The strain K599, also known as NCPPB 2659, is the causative agent of hairy root disease in a variety of plant species and was isolated from cucumber (*Cucumis sativus*) in a 1970s outbreak of hairy root disease (Mankin et al. 2007). The strain K599 has a combination of biovar 1 chromosomal background and rhizogenic plasmid and was assigned as *A. rhizogenes* in previous studies (Mankin et al. 2007) according to classification based on phytopathogenic properties that is no longer valid in taxonomy of agrobacteria. This strain harbors a root-inducing plasmid (pRi2659) and has been used to generate transgenic hairy root cultures and composite plants (Valdes Franco et al. 2016). In a recent paper by Singh et al. (2021), published after submission of our manuscript, strain K599 was classified as a novel genomospecies G21; however, our results clearly showed that this strain actually belonged to the genomospecies G20.

Based on core-genome phylogeny (Fig. 1), strain Rnr^T clustered with *Agrobacterium* strains ICMP 6402 and SBV_302_78_2. Although genome sequences of two latter strains were available in GenBank, their exact taxonomic position was not fully determined. ANI between the strain Rnr^T and strains ICMP 6402 and SBV_302_78_2 was 97.3 and 97.4%, respectively (Table 2), whereas DDH between the strain Rnr^T and two latter strains was 79.5 and 78.2%, respectively, which indicated that they belong to the same species. The strains ICMP 6402 and SBV_302_78_2 were isolated from grapevine and kiwifruit, respectively. A comprehensive ANI/dDDH analysis showed that these strains could not be assigned to any of the previously described species within *Rhizobiaceae*. As a result, these three strains were considered members of the same still-undescribed species.

Taking together this evidence, it is conceivable that the genomospecies G19 and G20, which have recently been described by Mafakheri et al. (2019), could be raised to the species level whereas each of the two new species include additional, thus far taxonomically undetermined *Agrobacterium* strains (Fig. 1). Hence, we propose *A. burrii* sp. nov. to encompass the G19 strains isolated from *Rosa* sp. in central Iran (Isfahan province) (i.e., Rnr^T, Rew, and Rnw) as well as the strains ICMP 6402 and SBV_302_78_2, whose whole genome sequences were retrieved from the GenBank database. We also propose *A. shirazense* sp. nov. to include G20 strains R13 and OT33^T isolated from *Rosa* sp. in Shiraz (Southern Iran) as well as the strains NCPPB 2655, MAFF 210266, 17-1007, 17-1008, and K599, whose whole genome sequences were retrieved from GenBank.

TABLE 2. Average nucleotide identity (lower diagonal) and digital DNA–DNA hybridization (upper diagonal) values among the strains sequenced in this study and a representative set of *Rhizobiaceae* strains selected based on the results of core genome-based phylogeny[a]

Strain	Former taxonomy	Proposed taxonomy	Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	G19	*Agrobacterium burrii*	Rnr^T	–	39.5	20.2	24.1	33.6	32.6	24.1	79.5*	78.2*	48.1	39.9	39.7	39.6	39.0	38.9	20.3	20.6
2	G20	*A. shirazense*	OT33^T	89.2	–	20.6	21.7	35.9	33.4	21.7	39.7	39.7	44.5	84.4*	82.4*	83.7*	82.5*	82.6*	20.5	21.0
3		*Rhizobium cellulosilyticum*	Ap1	76.1	76.6	–	20.2	20.6	20.6	20.0	20.6	20.1	20.5	20.9	20.6	20.7	20.7	20.7	71.7*	28.4
4		*A. rosae*	AE1	80.8	78.5	75.5	–	21.5	20.6	72.4*	22.0	21.3	21.4	21.6	21.2	21.4	21.3	21.3	20.2	20.4
5	G6		NCPPB 925	87.0	88.0	76.6	78.4	–	45.5	21.5	33.9	34.0	35.0	36.5	36.0	35.8	35.8	35.8	20.5	20.5
6	G8	"*A. fabrum*"	C58	86.8	87.1	75.8	77.2	91.0	–	20.8	32.7	32.3	33.0	32.2	33.9	33.7	33.3	33.2	21.0	21.0
7		*A. rosae*	NCPPB 1650^T	81.2	78.6	75.5	96.9*	77.8	76.2	–	21.6	21.2	21.4	21.8	21.3	21.4	21.4	21.4	19.8	20.1
8	*Agrobacterium* sp.	*A. burrii*	ICMP 6402	97.3*	89.6	74.0	77.0	87.0	86.6	76.9	–	79.3*	47.8	39.8	39.7	39.6	39.4	39.4	20.5	20.6
9	*Agrobacterium* sp.	*A. burrii*	SBV_302_78_2	97.4*	89.7	73.8	76.6	87.1	86.3	76.7	97.5*	–	65.5	39.7	39.6	39.6	39.3	39.3	20.3	20.7
10	*Agrobacterium* sp.		GBBC3284	91.7	91.1	74.1	76.6	87.4	86.7	76.6	91.7	91.8	–	44.1	45.0	46.1	44.1	44.0	20.5	20.6
11	*Agrobacterium* sp.	*A. shirazense*	K599	90.0	97.1*	74.3	77.1	87.8	72.7	77.2	90.0	89.9	91.4	–	84.2*	84.5*	84.5*	84.5*	20.4	20.7
12	*Agrobacterium* sp.	*A. shirazense*	MAFF210266	89.3	97.6*	74.2	76.7	87.8	87.0	76.8	89.6	89.4	91.5	97.1*	–	81.4*	81.4*	81.4*	20.4	20.9
13	*Agrobacterium* sp.	*A. shirazense*	NCPPB 2655	89.3	97.8*	74.2	76.8	87.7	87.1	76.8	89.5	89.5	91.6	97.1*	97.6*	–	82.7*	82.7*	20.5	20.9
14	*Agrobacterium* sp.	*A. shirazense*	17-1008	89.2	97.7*	74.2	76.7	87.9	87.0	77.0	89.4	89.4	90.7	96.9*	97.3*	97.5*	–	100.0*	20.5	20.7
15	*Agrobacterium* sp.	*A. shirazense*	17-1007	89.1	97.8*	74.2	76.9	87.8	86.9	76.8	89.3	89.3	90.7	97.1*	97.2*	97.5*	100.0*	–	20.5	20.7
16	*R. cellulosilyticum*		DSM 18291^T	74.89	74.3	96.3*	72.9	74.2	73.6	73.0	74.1	74.1	74.1	74.3	74.1	74.2	74.2	74.3	–	28.4
17	*R. smilacinae*		CCTCC AB 2013016^T	74.1	74.2	84.2	73.0	74.1	74.0	73.1	74.2	74.1	74.2	74.8	74.2	74.2	74.2	74.2	84.4	–

[a] A combination of average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) indices was used to designate a taxonomic status to a given phylogenetic clade where the strain Rnr^T as well as the *Agrobacterium* sp. strains ICMP 6402 and SBV_302_78_2 were members of the same species and designated as *A. burrii* sp. nov., whereas the strain OT33^T along with the strains NCPPB 2655, MAFF 210266, 17-1007, 17-1008, and K599 were named as *A. shirazense* sp. nov. Asterisks indicate the ANI and dDDH values higher than the accepted threshold for the definition of prokaryotic species.

DESCRIPTION OF *A. BURRII* SP. NOV.

A. burrii (burr'i.i. N.L. gen. n. *burrii*) was named in honor of Thomas J. Burr, a prominent plant pathologist at Cornell University (Ithaca, NY) who is well-known for his outstanding accomplishments in research on agrobacteria.

General characteristics of the species are similar to those described for the genus *Agrobacterium* (Conn 1942). Colonies of the type strain Rnr^T on yeast mannitola agar (YMA) medium are translucent, creamy white, circular, and glistening and are 1 to 2 mm in diameter after incubation for 2 days at 28°C. The strain Rnr^T produces 3-ketolactose from lactose and can grow in the presence of 2.0% (wt/vol) NaCl but is negative in the use of citrate. The strain Rnr^T is also able to use adonitol, D-cellobiose, D-fructose, D-maltose, D-mannitol, D-raffinose, D-sorbitol, inositol, L-arabinose, and L-rhamnose. However, it is unable to use dulcitol and xylose. The strain Rnr^T is pathogenic on tomato and sunflower seedlings as well as carrot root discs. *A. burrii* sp. nov. can be differentiated from other species of the genus *Agrobacterium* based on OGRIs calculations (ANIb and dDDH). DNA G+C content of the type strain is 58.9%. Its approximate genome size is 6.08 Mbp. The type strain Rnr^T = CFBP 8705^T = DSM 112541^T was isolated from crown gall of *Rosa* sp. at Najafabad County in Isfahan Province, Iran. Whole-genome shotgun sequence of the strain Rnr^T has been deposited at the NCBI GenBank under accession number JAFLNA000000000.

DESCRIPTION OF *A. SHIRAZENSE* SP. NOV.

A. shirazense (shi.ra.zen'se. N.L. neut. adj. *shirazense*) was named in honor of Shiraz, the capital of Fars province in Southern Iran, where the type strain was isolated.

General characteristics of the species are similar to those described for the genus *Agrobacterium* (Conn 1942). Colonies of the type strain $OT33^T$ on YMA medium are translucent, creamy white, circular, and glistening and are 1 to 2 mm in diameter after incubation for 2 days at 28°C. The strain $OT33^T$ produces 3-ketolactose from lactose, uses citrate, and can grow in the presence of 2.0% (wt/vol) NaCl. It is able to use adonitol, D-cellobiose, D-fructose, D-maltose, D-mannitol, D-raffinose, D-sorbitol, dulcitol, and xylose. The strain $OT33^T$ was pathogenic on neither tomato nor sunflower plants under greenhouse conditions. *A. shirazense* sp. nov. can be differentiated from other species of the genus *Agrobacterium* based on OGRIs calculations (ANIb and dDDH). DNA G+C content of the type strain is 59.9%. Its approximate genome size is 5.51 Mbp. The type strain $OT33^T$ = CFBP 8901^T = DSM 112540^T was isolated from crown gall of *Rosa* sp. in Shiraz, Fars Province, Southern Iran. Whole-genome shotgun sequence of the strain $OT33^T$ has been deposited at the NCBI GenBank under accession number JAFLMZ000000000.

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