

# *Agrobacterium cavarae* sp. nov., isolated from maize (*Zea mays* L.) roots

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## Abstract

A bacterial strain designated as RZME10<sup>T</sup> was isolated from a *Zea mays* L. root collected in Spain. Results of analysis of the 16S rRNA gene sequence showed that this strain belongs to the genus *Agrobacterium* with *Agrobacterium larrymoorei* ATCC 51759<sup>T</sup> being the most closely related species with 99.9% sequence similarity. The similarity values of the *rpoB*, *recA*, *gyrB*, *atpD* and *glnII* genes between strain RZME10<sup>T</sup> and *A. larrymoorei* ATCC 51759<sup>T</sup> were 93.5, 90.0, 88.7, 87.9 and 90.1%, respectively. The estimated average nucleotide identity using BLAST and digital DNA–DNA hybridization values between these two strains were 80.4 and 30.2%, respectively. The major fatty acids of strain RZME10<sup>T</sup> are those from summed feature 8 (C<sub>18:1</sub> ω6c/C<sub>18:1</sub> ω7c) and C<sub>16:0</sub>. Pathogenicity tests on tomato and carrot roots showed that strain RZME10<sup>T</sup> was not able to induce plant tumours. Based on the results of genomic, chemotaxonomic and phenotypic analyses, we propose that strain RZME10<sup>T</sup> represents a novel species named *Agrobacterium cavarae* sp. nov. (type strain RZME10<sup>T</sup>=CECT 9795<sup>T</sup>=LMG 31257<sup>T</sup>).

## INTRODUCTION

The genus *Agrobacterium* comprises Gram-stain-negative motile aerobic rods that form convex, circular, smooth, non-pigmented to light beige colonies. In media containing carbohydrates as carbon sources, members of the genus *Agrobacterium* produce acids and copious extracellular polysaccharide slime [1]. This genus currently contains pathogenic species able to induce plant tumours or hairy roots and also non-pathogenic species isolated from soil and different plant-related sources [2, 3]. Although some of these species were originally isolated from legume nodules, none of them have been isolated from cereals to date [2, 3].

Taking this into account, and considering the importance of the genus *Agrobacterium* in plant diseases, in the present work we characterize a strain named RZME10<sup>T</sup> isolated from the root of a maize plant [4], which was closely related to *Agrobacterium larrymoorei*, a species containing tumourigenic strains isolated from tumours of *Ficus benjamina* [5]. Based on the genotypic, chemotaxonomic and phenotypic characteristics of strain RZME10<sup>T</sup>, we propose its classification as

representing a novel species named *Agrobacterium cavarae* sp. nov.

## ISOLATION AND ECOLOGY

Strain RZME10<sup>T</sup> was isolated from a root of *Zea mays* L. growing in soil from Riego de la Vega (León, NW Spain, 42° 23' 21" N, 5° 58' 56" W), where this cereal is widely cultivated, in the course of a study on maize root endophytes [4]. For isolation, the roots were surface-disinfected with 70% (v/v) ethanol (1 min), 2% NaClO (w/v) (3 min) and 70% (v/v) ethanol (30 s) and then they were rinsed five times with sterile distilled water and crushed in 10 ml sterile PBS, pH 7. Serial decimal dilutions were performed and aliquots of 0.1 ml from each dilution were inoculated on trypticase soy agar (TSA; Difco, Becton Dickinson) for incubation at 48 h and 28 °C. In parallel, some of the disinfected root samples were incubated in the same medium to ensure their complete external disinfection and no growth was observed around these roots.

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**Keywords:** *Agrobacterium*; endophyte; maize; Spain; *Zea mays* L.

**Abbreviations:** ANIb, average nucleotide identity based on BLAST; dDDH, digital DNA–DNA hybridization; GBDP, genome BLAST distance phylogeny; MLSA, multilocus sequence analysis; TSA, trypticase soy agar; TY, tryptone yeast medium; YMA, yeast mannitol agar.

The GenBank accession numbers for the 16S rRNA gene and genome sequences are MK940276 and SISF00000000, respectively.

One supplementary table and two supplementary figures are available with the online version of this article.

## PHYLOGENETIC CHARACTERIZATION

The 16S rRNA gene was amplified and sequenced in previous work [6] by the Sequencing DNA Service (NUCLEUS) at Salamanca University (Spain). The obtained sequence was compared with those from GenBank using the BLASTN programme [7]. MLSA was based on the concatenated sequences of five housekeeping genes: *rpoB*, *recA*, *gyrB*, *atpD* and *glnII*. These sequences as well as that of the *telA* gene were extracted from the genome of strain RZME10<sup>T</sup> and compared with those of the remaining species of the genus *Agrobacterium* available in GenBank. The sequences were aligned using the CLUSTAL\_W programme [8]. The phylogenetic distances were calculated according to Kimura's two-parameter model [9]. The phylogenetic trees were inferred using the neighbour-joining model [10] and MEGA 7.09 [11] was used for all phylogenetic analyses.

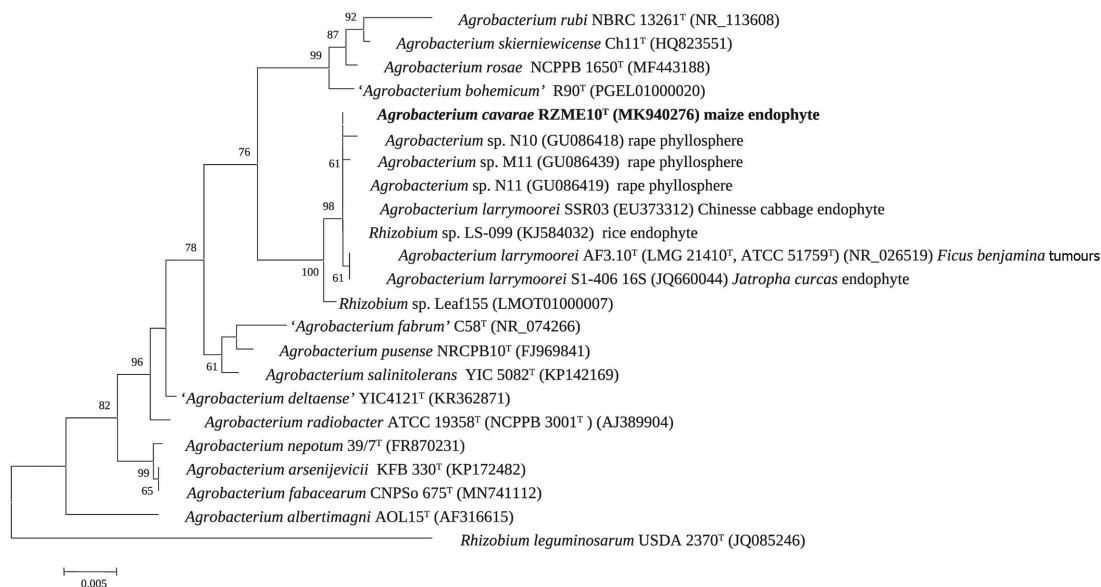
The 16S rRNA gene of strain RZME10<sup>T</sup> showed 99.9% similarity with respect to that of the type strain of *A. larrymoorei*, which is its closest-related species. The remaining species of the genus *Agrobacterium* showed values slightly higher or lower than 98% similarity with respect to strain RZME10<sup>T</sup>. High similarity values were also presented by several strains isolated from plant-related sources, some of them named as *Rhizobium* species, such as strain LS-099, which is a rice endophyte [12], and strain Leaf155, which was isolated from a leaf of *Arabidopsis thaliana* [13]. The results of the 16S rRNA gene analysis showed that these two strains belong to the genus *Agrobacterium* and that strain SSR03, endophytic of Chinese cabbage [14], is closer to the new species than to *A. larrymoorei* (Fig. 1). However, for most of these strains, except for Leaf155, only sequences of 16S rRNA genes are available in GenBank and therefore they cannot be assigned to

the new proposed species taking into account the limitations of this gene for *Agrobacterium* species differentiation, as will be shown in this study. Since the genome of strain Leaf155 is available in GenBank, it will be compared with those of the type strains of the *Agrobacterium* species.

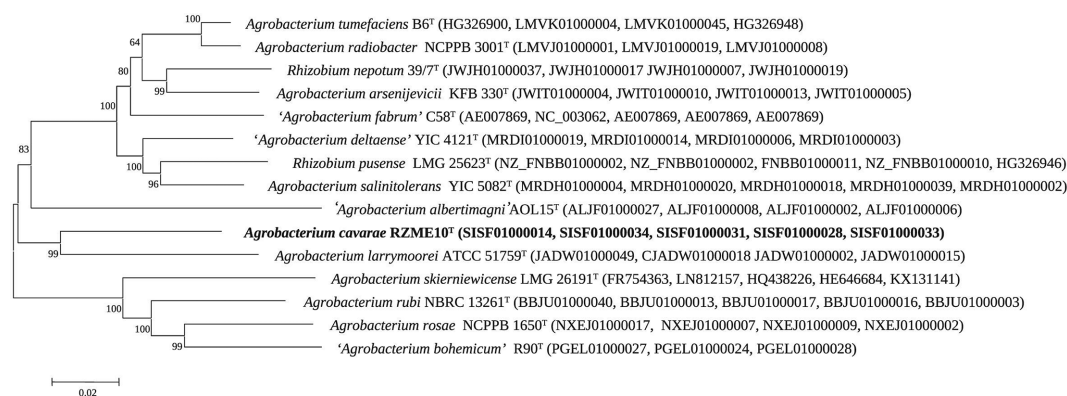
Strain RZME10<sup>T</sup> formed an independent cluster within the genus *Agrobacterium* together with *A. larrymoorei* AF3.10<sup>T</sup>=ATCC 51759<sup>T</sup> in both 16S rRNA gene analysis (Fig. 1) and MLSA based on *rpoB*, *recA*, *gyrB*, *atpD* and *glnII* genes (Fig. 2). Nevertheless, the sequence similarity values of these genes between RZME10<sup>T</sup> and *A. larrymoorei* ATCC 51759<sup>T</sup> were 93.5, 90.0, 88.7, 87.9 and 90.1%, respectively, which are lower than those found among other species of *Agrobacterium* (Fig. 2). The results of the phylogenetic analysis of the *telA* gene, which codifies a protelomerase involved in the maintenance of the linear chromid [15], showed that strain RZME10<sup>T</sup> also clustered with its closest relative *A. larrymoorei* AF3.10<sup>T</sup> with 81.7% similarity. The remaining type strains of *Agrobacterium* species were phylogenetically divergent from strain RZME10<sup>T</sup> with similarity values lower than 80% (Fig. S1, available in the online version of this article).

## GENOME FEATURES

The genomic DNA from pure culture of strain RZME10<sup>T</sup> was purified using the DNeasy UltraClean Microbial DNA Isolation Kit (Qiagen) following manufacturer's protocol. Sequencing, upon preparation of pair-end libraries, was performed on the Illumina MiSeq sequencing platform (2×250 bp). Sequencing data was assembled using Velvet 1.2.10 [16]. The draft genome sequence of strain RZME10<sup>T</sup>



**Fig. 1.** Neighbour-joining phylogenetic rooted tree based on 16S rRNA gene sequences (1380 nt) showing the taxonomic location of *Agrobacterium cavae* RZME10<sup>T</sup> within the genus *Agrobacterium*. Bootstrap values calculated for 1000 replications are indicated. Bar, 5 nt substitution per 1000 nt. Accession numbers from GenBank are given in parentheses.



**Fig. 2.** Neighbour-joining phylogenetic tree based on *rpoB*, *recA*, *gyrB*, *atpD* and *glnII* concatenated gene sequences (4110 nt) showing the taxonomic location of *Agrobacterium cavarae* RZME10<sup>T</sup> within the genus *Agrobacterium*. Bootstrap values calculated for 1000 replications are indicated. Bar, 2 nt substitution per 100 nt. Accession numbers from GenBank are given in parentheses.

was deposited in DDBJ/EMBL/GenBank under Bioproject PRJNA523189 (accession number SISF00000000). The genome characteristics are shown in Table S1. Average nucleotide identity using BLAST (ANIb) was calculated with the JSpecies server [17, 18] (<http://imedea.uib-csic.es/jspecies/>). Digital DNA–DNA hybridization (dDDH) values were calculated using the Genome-to-Genome Distance Calculator web service from the DSMZ (GGDC 2.1) [19] (<http://ggdc.dsmz.de/ggdc.php/>). These values were calculated using the formula 2 at the GGDC website [20] because it is the only function appropriate to analyse draft genomes [21]. The ANIb and dDDH values were calculated for the type strains of *Agrobacterium* species whose genomes are currently available and the values found between RZME10<sup>T</sup> and *A. larrymoorei* ATCC 51759<sup>T</sup> were 80.4 and 30.0%, respectively (Table 1). These values are far lower than the threshold values recommended for bacterial species differentiation [22] and, therefore, strain RZME10<sup>T</sup> belongs to a new species within the genus *Agrobacterium*. The ANIb and dDDH values between the genome sequences of strains RZME10<sup>T</sup> and Leaf155 were 95.79 and 68.30%, respectively (Table 1). These values are also lower than the threshold values recommended for species differentiation (95–96% for ANI and lower than 70% for dDDH) [22, 23] and therefore strain Leaf155 would represent a sister species to *A. cavarae*.

The G+C content of strain RZME10<sup>T</sup> calculated from the genome was 58.3mol% (Table S1), which falls within the range reported for the genus *Agrobacterium* [1].

Phylogenomic analysis was conducted with the Type Strain Genome Server [19]. This web-server tool employs the genome BLAST distance phylogeny method (GBDP) [24] to compare whole genome sequences at nucleotide level, allowing calculation of dDDH values and reconstructing the phylogram. The GBDP phylogenomic tree confirmed the phylogenetic position of strain RZME10<sup>T</sup> derived from the 16S rRNA gene analysis and MLSA showing that this strain clustered with the type strain of *A. larrymoorei* ATCC 51759<sup>T</sup> (Fig. 3).

Annotation was done using the SEED viewer and the RAST 2.0 server (Rapid Annotation using Subsystem Technology) [25, 26] and the NCBI Prokaryotic Genome Annotation Pipeline (PGAP; [www.ncbi.nlm.nih.gov/genome/annotation\\_prok/](http://www.ncbi.nlm.nih.gov/genome/annotation_prok/)) [27, 28]. The differences in gene composition of strain RZME10<sup>T</sup>, *A. larrymoorei* ATCC 51759<sup>T</sup> and *A. radiobacter* NCPPB 3001<sup>T</sup> are shown in Table 2. Some differences have been detected in hydrolytic activities of the analysed strains.  $\alpha$ -Amylase was detected in *A. larrymoorei* ATCC 51759<sup>T</sup> and *A. radiobacter* NCPPB 3001<sup>T</sup> but not in strain RZME10<sup>T</sup>.  $\beta$ -Galactosidase and endo-1,4- $\beta$ -xylanase (EC 3.2.1.8) were found in *A. radiobacter* NCPPB 3001<sup>T</sup>, but were absent in the other strains. The opine oxidase cluster was found in *A. larrymoorei* ATCC 51759<sup>T</sup> and *A. radiobacter* NCPPB 3001<sup>T</sup> but not in strain RZME10<sup>T</sup>. Isochorismate synthase and salicylate synthase have been annotated in strain RZME10<sup>T</sup>, however, the former was not detected in *A. larrymoorei* ATCC 51759<sup>T</sup> and the latter was not detected in *A. radiobacter* NCPPB 3001<sup>T</sup>. Both genes are related to the modulation of plant defence response [29–31]. The T-DNA region genes have not been found in RZME10<sup>T</sup> and *A. radiobacter* NCPPB 3001<sup>T</sup>, but they have been annotated in *A. larrymoorei* ATCC 51759<sup>T</sup>.

## PHYSIOLOGY AND CHEMOTAXONOMY

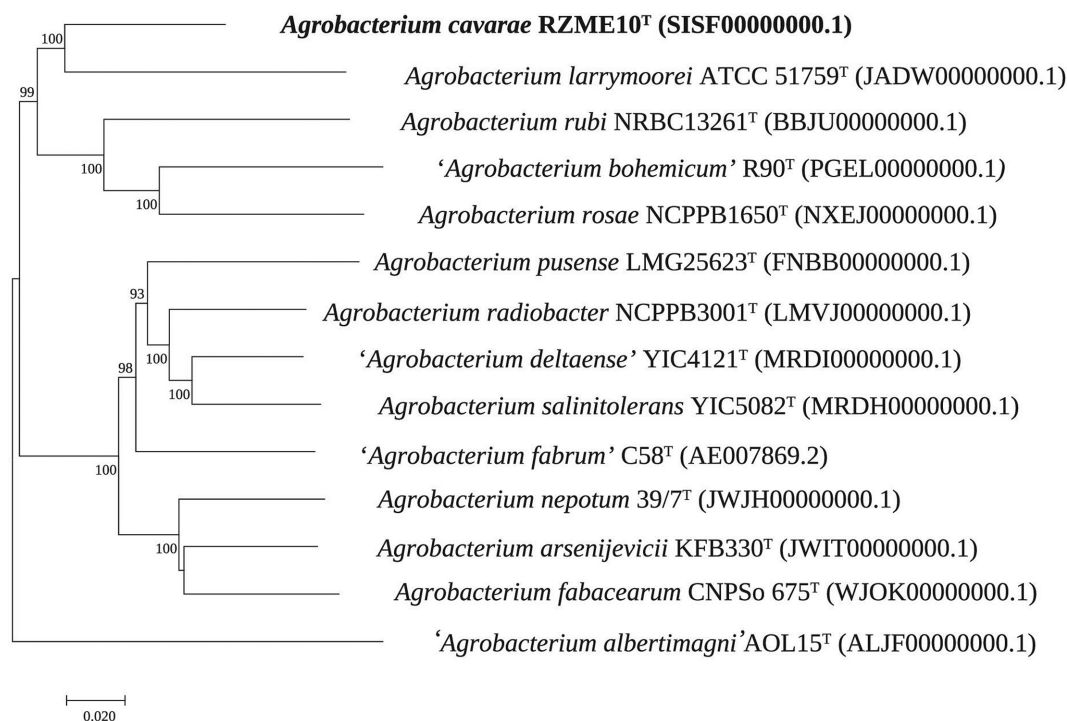
Phenotypic characterizations were performed using the API ID32GN and API 20NE systems (bioMérieux) under the conditions indicated by the manufacturer and the results were read after 72 h incubation at 28 °C. Growth temperature range was determined by incubating cultures in Yeast Mannitol Agar (YMA) medium [32] at 4, 15, 28, 37 and 45 °C. Growth pH range was determined in the same medium with final pH 4.0, 6, 7, 8, 9 and 10. PCA buffer (Na<sub>2</sub>HPO<sub>4</sub> 0.4M and citric acid 0.2M) was used to adjust the pH from pH 4 to 6, phosphate buffer (Na<sub>2</sub>HPO<sub>4</sub> 0.2M and NaH<sub>2</sub>PO<sub>4</sub> 0.2M) was used for pH 7 and TE buffer 0.2M was used for pH 8 and 9. Salt tolerance was tested in the same medium containing 0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4% (w/v) NaCl. Catalase production was assayed by using 0.3% hydrogen peroxide with one colony taken from

**Table 1.** Pairwise dDDH and ANIb values among the type strains of *Agrobacterium* species

Strains: 1, ZME10<sup>T</sup> (SISF00000000.1); 3, *Agrobacterium* sp. Leaf155 (LMOT00000000.1); 3, *Agrobacterium larrymoorei* ATCC 51759<sup>T</sup> (JADW00000000.1); 4, *Agrobacterium rubi* NRBC13261<sup>T</sup> (BBJU00000000.1); 5, *Agrobacterium radiobacter* NCPPB3001<sup>T</sup> (LMVJ00000000.1); 6, *Agrobacterium albertimagni* AOL15<sup>T</sup> (ALJF00000000.1); 7, *Agrobacterium fabrum* C58<sup>T</sup> (AE007869.2); 8, *Agrobacterium pusense* LMG25623<sup>T</sup> (FNBB00000000.1); 9, *Agrobacterium nepotum* 391/7<sup>T</sup> (UWJH00000000.1); 10, *Agrobacterium arseniivicii* KFB330<sup>T</sup> (JWIT00000000.1); 11, *Agrobacterium deltaense* YIC4121<sup>T</sup> (MRDI00000000.1); 12, *Agrobacterium salinitolerans* YICS082<sup>T</sup> (MRDH00000000.1); 13, *Agrobacterium bohemicum* R90<sup>T</sup> (PGEL00000000.1); 14, *Agrobacterium rosae* NCPPB1650<sup>T</sup> (NXEJ00000000.1); 15, *Agrobacterium fabacearum* CNPSo 675<sup>T</sup> (WJOK00000000.1). ANIb values are in the bottom left and dDDH values in the top right. Data are in percentages.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	*	68.30	24.10	21.90	22.10	20.50	22.10	22.10	22.00	22.10	22.10	22.00	21.30	21.80	22.40
2	95.79	*	24.10	21.90	22.10	20.50	22.30	22.10	22.00	22.10	22.40	22.00	21.30	21.80	22.40
3	80.76	79.86	*	22.50	22.20	20.10	22.20	21.90	22.00	21.70	22.30	22.10	21.80	22.40	21.70
4	77.82	76.92	78.05	*	21.50	19.80	23.80	21.40	22.10	23.70	21.50	21.20	25.90	27.00	21.30
5	77.36	76.62	76.88	76.25	*	20.40	36.30	34.10	33.90	34.20	44.10	40.40	20.80	21.30	33.30
6	73.19	72.66	72.27	72.00	72.94	*	20.70	20.50	20.70	20.60	20.60	20.50	19.90	20.30	20.70
7	77.31	76.72	76.72	77.52	87.93	72.97	*	31.80	32.00	33.10	35.70	35.90	21.20	21.50	31.40
8	77.04	76.33	76.48	75.90	87.04	72.82	85.95	*	29.60	29.80	35.10	33.50	20.80	21.40	28.80
9	77.39	76.60	76.72	76.72	86.69	72.85	85.84	84.76	*	42.40	33.80	31.90	21.00	21.50	38.80
10	77.24	76.57	76.76	77.60	86.91	72.56	86.51	84.80	89.82	*	33.80	32.00	20.80	21.30	41.30
11	77.54	76.85	77.11	76.33	90.61	73.15	87.55	87.45	86.74	86.91	*	46.80	21.10	21.70	32.20
12	77.35	76.66	76.77	76.11	89.57	72.97	87.90	86.72	86.00	85.80	91.38	*	20.70	21.40	30.90
13	77.32	76.40	77.23	81.29	75.75	71.67	75.59	75.36	75.85	75.69	75.99	75.69	*	30.80	21.00
14	77.62	76.87	77.85	82.52	76.28	71.84	76.11	75.87	76.32	76.14	76.24	76.13	85.08	*	21.40
15	77.14	76.36	76.51	75.92	86.59	72.57	85.65	84.13	88.63	89.45	86.03	85.40	75.40	75.88	*





**Fig. 3.** Whole genome based phylogenomic tree reconstructed with the GBDP tool and retrieved from the TYGS website. The tree was inferred with FastME 2.1.6.1 from GBDP distances calculated from genome sequences. The branch lengths are scaled in terms of GBDP distance formula d5. The numbers above branches are GBDP pseudo-bootstrap support values >60% from 100 replications, with an average branch support of 85.7%.

the Tryptone Yeast (TY) plates. Oxidase activity was detected by using *N,N,N',N'*-tetramethyl-1,4-phenylenediamine dihydrochloride. To test the natural antibiotic resistance, the disc diffusion method on YMA medium was used. The discs contained the following antibiotics: ampicillin (2 µg), erythromycin (2 µg), ciprofloxacin (5 µg), penicillin (10 IU), polymyxin (300 IU), cloxacillin (1 µg), tetracycline (30 µg), gentamycin (10 µg), cefuroxime (30 µg) or neomycin (5 µg) (Becton Dickinson, BBL). *A. larrymoorei* LMG 21410<sup>T</sup> (ATCC 51759<sup>T</sup>) and *A. radiobacter* ATCC 19358<sup>T</sup> (=NCPPB 3001<sup>T</sup>) were included in the phenotypic study as references. Pathogenicity assays on tomato plants and discs of carrot roots were performed according to methodologies previously described using *A. larrymoorei* LMG 21410<sup>T</sup> (ATCC 51759<sup>T</sup>) and *A. tumefaciens* ATCC 23308<sup>T</sup> as references [33, 34].

Phenotypic characteristics of the new species are reported below in the species description and the differences with respect to the closest species are recorded in Table 3. Strain RZME10<sup>T</sup> differed from its closest-related species in growth at 5 °C and in presence of 4% NaCl and in assimilation as sole carbon and energy sources of several compounds. Strain RZME10<sup>T</sup> was sensitive to ciprofloxacin, gentamycin, neomycin and tetracycline and resistant to ampicillin, cefuroxime, cloxacillin and erythromycin. It was weakly sensitive to penicillin and polymyxin B. The results of pathogenicity assays showed that strain RZME10<sup>T</sup> does not induce tumours

in tomato or in carrot roots discs (Fig. S2), in agreement with the absence of the genes from T-DNA region in its genome.

The cellular fatty acids were analysed by using the Microbial Identification System (MIDI; Microbial ID) Sherlock 6.1 and the library RTSBA6 according to the technical instructions provided by this system [35]. Strain RZME10<sup>T</sup>, *A. larrymoorei* LMG 21410<sup>T</sup> and *A. radiobacter* NCPPB 3001<sup>T</sup> were cultured aerobically on TY plates [36] at 28 °C and cells were collected during the late-exponential phase of growth. The obtained results showed that the major fatty acids of *A. cavarae* RZME10<sup>T</sup> are those from summed feature 8 (*C*<sub>18:1</sub> ω6c/*C*<sub>18:1</sub> ω7c) and *C*<sub>16:0</sub> as in the other analysed *Agrobacterium* strains (Table 4). The most relevant difference between strain RZME10<sup>T</sup> and *A. larrymoorei* LMG 21410<sup>T</sup> was the amount of *C*<sub>19:0</sub> cyclo ω8c which was higher in the latter strain.

Collectively, the data obtained in this study showed that strain RZME10<sup>T</sup> belongs to a new species of the genus *Agrobacterium*. Several new species of this genus have been originally isolated from root legume nodules, such as *A. deltaense*, *A. salinitolerans* and *A. fabacearum* [37–39], but *A. cavarae* is the first one isolated from the roots of *Zea mays* L. Although positive effects of some endophytic *Agrobacterium* strains on the growth of legumes have been reported [40, 41], further studies are necessary to analyse the role of the new species *A. cavarae* in the growth and health of *Zea mays* L. plants.

**Table 2.** Comparison of the presence and absence of selected genes in the genomes of the strain RZME10<sup>T</sup> (SISF00000000.1), its related type strain *Agrobacterium larrymoorei* ATCC 51759<sup>T</sup> (JADW00000000.1) and the type strain of *Agrobacterium radiobacter* NCPPB 3001<sup>T</sup> (LMVJ00000000.1), which is the type species of genus *Agrobacterium*

Strains: 1, RZME10<sup>T</sup>; 2, *A. larrymoorei* ATCC 51759<sup>T</sup>; 3, *A. radiobacter* NCPPB3001<sup>T</sup>. +, Present; –, absent.

Genes	1	2	3
<b>Genes encoding hydrolytic enzymes</b>			
α-Amylase (EC 3.2.1.1)	–	+	+
Endo-1,4-β-xylanase (EC 3.2.1.8)	–	–	+
Opine oxidase cluster	–	+	+
Periplasmic nitrate reductase (EC 1.7.99.4)	+	+	–
α-Galactosidase (EC 3.2.1.22)	Two genes	One gene	Two genes
β-Galactosidase (EC 3.2.1.23)	–	–	+
Arginine deiminase (EC 3.5.3.6)	–	+	–
<b>Other genes</b>			
Isochorismate synthase	+	–	+
Isochorismatase (EC 3.3.2.1)	Two genes	Two genes	Three genes
Circadian clock protein <i>KaiC</i>	+	–	–
Salicylate synthetase (EC 5.4.4.2)	+	+	–
Potassium efflux system <i>kefA</i>	–	–	+
Queuosine biosynthesis ATPase <i>QueC</i>	–	+	–
Beta-ketoacyl-ACP synthase III	–	–	+
Aminopeptidase P	Three genes	–	Three genes
Xylose ABC transporter <i>XylF</i>	Three genes	One gene	One gene
T-DNA region genes	–	+	–

## DESCRIPTION OF *AGROBACTERIUM CAVARAE* SP. NOV.

*Agrobacterium cavarae* (ca.va'rae N.L. masc. gen. n. *cavarae*, to honour Dr. Fidriano Cavara, who first isolated a bacterium causing galls in grapevines).

Cells are Gram-stain-negative, aerobic, motile and rod-shaped. Colonies on YMA are white, circular and convex with diameter of 1 mm within 48 h at 28 °C. Catalase- and oxidase-positive. It grows at 5–40 °C and optimally at 28 °C and from pH 5 to 8 with optimum growth at pH 7. It grows with up to 4% NaCl. Nitrate reduction, arginine dehydrolase and gelatinase are negative, and urease and β-galactosidase are positive. Aesculin hydrolysis is positive. Assimilation of D-glucose, L-arabinose, L-rhamnose, D-ribose, D-mannose,

**Table 3.** Phenotypic characteristics of the strain RZME10<sup>T</sup>, its related type strain of *Agrobacterium larrymoorei* and the type strain of *Agrobacterium tumefaciens*, which is the type species of genus *Agrobacterium*

Strains: 1, RZME10<sup>T</sup>; 2, *A. larrymoorei* LMG 21410<sup>T</sup>; 3, *A. radiobacter* ATCC 19358<sup>T</sup>. Data are from this study. +, Positive; –, negative; w, weak.

Characteristics	1	2	3
Growth at:			
5 °C	+	–	+
4% NaCl	+	–	–
Assimilation of:			
Salicin	+	–	+
Sorbitol	+	–	+
Propionate	–	–	+
2-Keto-gluconate	–	–	+
5-Keto-gluconate	+	–	+
Citrate	+	w	–
3-Hydroxl-butyrate	–	–	–
4-Hydroxibenzoate	–	+	w
Phenyl-acetate	–	+	–

mannitol, *N*-acetyl-glucosamine, inositol, sucrose, maltose, gluconate, malate, citrate, salicin, melibiose, L-fucose, D-sorbitol, L-alanine, 5 keto-gluconate, L-histidine and L-proline is positive. Assimilation of caprate, adipate,

**Table 4.** Cellular fatty acid composition of the strain RZME10<sup>T</sup>, its related type strain of *Agrobacterium larrymoorei* and the type strain of *Agrobacterium tumefaciens*, which is the type species of genus *Agrobacterium*

Strains: 1, *A. cavarae* RZME10<sup>T</sup>; 2, *A. larrymoorei* LMG 21410<sup>T</sup>; 3, *A. radiobacter* ATCC 19358<sup>T</sup>. Fatty acids present in amounts lower than 1% in all species are not shown. Data are from this study. ND, not detected.

Fatty acid	1	2	3
C <sub>16:0</sub>	9.4	11.0	9.0
C <sub>18:0</sub>	1.1	0.5	0.5
C <sub>16:0</sub> 3OH	2.7	3.0	5.6
C <sub>18:1</sub> ω7c 11-methyl	1.2	0.7	0.6
C <sub>19:0</sub> cyclo ω8c	2.0	6.8	10.7
C <sub>19:0</sub> 10-methyl	0.7	0.3	ND
Summed feature 2*	5.6	5.5	6.6
Summed feature 3†	2.7	3.7	1.0
Summed feature 8‡	74.6	66.1	64.6

\*Summed feature 2, C<sub>14:0</sub> 3OH/C<sub>16:1</sub> iso l.

†Summed feature 3, C<sub>16:1</sub> ω7c/C<sub>16:1</sub> ω6c.

‡Summed feature 8, C<sub>18:1</sub> ω7c/C<sub>18:1</sub> ω6c).

phenylacetate, itaconate, suberate, malonate, propionate, valerate, glycogen, 3-hydroxy-butyrate, 2-keto-gluconate and 3- and 4-hydroxy-benzoate is negative. Acetate, D,L-lactate and L-serine are weakly assimilated. The major fatty acids are those from summed feature 8 ( $C_{18:1} \omega 6c/C_{18:1} \omega 7c$ ) and  $C_{16:0}$ . The G+C content is 58.3mol%.

The type strain, RZME10<sup>T</sup> (=CECT 9795<sup>T</sup>=LMG 31257<sup>T</sup>), was isolated from a root of *Zea mays* L. The draft genome sequence was deposited in DDBJ/EMBL/GenBank under the Bioproject PRJNA523189 (accession number SISF00000000) and the 16S rRNA gene under the accession number MK940276.

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#### Author contributions

Conceptualization: E. V. Data curation: P. G. F. Formal Analysis: J. D. F. F., E. M., M. H. R. B. Funding acquisition: A. P., E. V. Investigation J. D. F. F., E. M., M. H. R. B. Methodology J. D. F. F., E. M., E. V. Project administration A. P., E. V. Supervision: P. G. F., A. P., E. V. Writing – original draft J. D. F. F., E. M. Writing – review and editing M. H. R. B., A. P., P. G. F., E. V.

#### Conflicts of interest

The authors declare that there are no conflicts of interest.

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