NOTE

Agrobacterium larrymoorei* sp. nov., a pathogen isolated from aerial tumours of *Ficus benjamina

Hacène Bouzart and Jeffrey B. Jones

Department of Plant Pathology, University of Florida, PO Box 110680, Gainesville, FL 32611-0680, USA

Author for correspondence: Jeffrey B. Jones. Tel: +1 352 392 7244. Fax: +1 352 392 6532. e-mail: jbjones@ufl.edu

Tumorigenic *Agrobacterium* strains isolated from tumours growing on pruned branches of *Ficus benjamina* have previously been shown to have unique opine metabolism and sufficient 16S rRNA sequence differences to suggest that they belong to a new species. DNA–DNA hybridization results confirmed that these strains represent a new species and *Agrobacterium larrymoorei* sp. nov. (type strain ATCC 51759^T = CFBP 5473^T = NCPPB 4096^T) is proposed as the name for the species.

Keywords: taxonomy, bacteria, fig, crown gall

The genus *Agrobacterium* Conn, a member of the *Rhizobiaceae*, is included in the α -2 subclass of *Proteobacteria* as determined by ribosomal characteristics (Woese *et al.*, 1984). According to *Bergey's Manual of Systematic Bacteriology* (Kerstens & De Ley, 1984) and Skerman *et al.* (1980), *Agrobacterium* can be divided into four species. The non-pathogenic agrobacteria were assigned to *Agrobacterium radiobacter*, agrobacteria causing crown gall were assigned to *Agrobacterium tumefaciens*, the agents of cane gall on *Rubus* spp. were assigned to *Agrobacterium rubi* and the bacteria that induce root proliferation were assigned to *Agrobacterium rhizogenes*. Epithets for these species were based on phytopathogenic properties carried on plasmids. The nomenclature and taxonomic structure of these species did not correlate with the chromosome-encoded phenotypic and genotypic characteristics. According to phenotypic and genetic characteristics, most agrobacteria (except *A. rubi*) cluster into three distinct chromosomal groups or biovars (Kerstens & De Ley, 1984). Although biovar is a term used to designate variation at the infra-subspecific level, it was used at the time to distinguish physiological groups within each *Agrobacterium* species. Later, biovars 1, 2 and 3 were elevated to *A. tumefaciens*, *A. rhizogenes* and *Agrobacterium vitis*, respectively (Bouzar, 1994; Holmes & Roberts, 1981; Kersters & De Ley, 1984; Ophel & Kerr, 1990; Judicial Commission of the International Committee on Systematic Bacteriology, 1995; Sawada *et al.*, 1993).

In 1991, *Agrobacterium* strains were isolated from tumours growing on branches and pruning wounds of weeping fig trees (*Ficus benjamina* L.) grown in a Florida nursery (Bouzar *et al.*, 1995). These weeping fig (hereafter referred to as fig) strains, which were designated AF, differed from *A. tumefaciens*, *A. rhizogenes*, *A. vitis* and *A. rubi* in terms of their differential oxidation of carbon substrates and fatty acid content. The AF strains also produced three unusual opines: chrysopine, *N*-1-deoxy-D-fructosyl-L-glutamine and *N*-1-deoxy-D-fructosyl-5-oxo-L-proline (Bouzar *et al.*, 1995). Phylogenetic relationships based on sequence analysis of the *rrs* gene (i.e. 16S rRNA gene) revealed that a representative fig strain (AF3.10^T) clustered most closely with *A. rubi* and *A. tumefaciens*, but clustered more distantly from *A. vitis* and was remote from *A. rhizogenes* (Bouzar *et al.*, 1995). In that study, the sequence was sufficiently different from that of the two most closely related species to suggest that the fig strain may be a novel species. These results led us to perform the present study, to determine the levels of DNA homology that exist between the fig strain and the type strains of other plant- and soil-borne *Agrobacterium* species.

DNA–DNA relatedness was assessed in a microtitre plate and determined by the fluorometric assay of Ezaki *et al.* (1989) with minor modifications (Jones *et al.*, 2000). Large molecular mass DNA was extracted from the type strains of *A. rhizogenes*, *A. rubi*, *A. tumefaciens* and *A. vitis* and strains isolated from aerial tumours of *F. benjamina* (Table 1). DNA extraction and purification were performed following Marmur's procedure as described by Johnson (1994). The DNA

† Present address: Sakata Seed America Inc., 105 Boronda Rd, Salinas, CA 93907-1830, USA.

Table 1. *Agrobacterium* strains used in this study

Species	Strain	Other designation	Source*
<i>A. rhizogenes</i>	AR13 ^T	ATCC 11325 ^T	ATCC
<i>A. rubi</i>	ARU2 ^T	TR3 ^T (= ATCC 13335 ^T)	L. W. Moore
<i>A. tumefaciens</i>	AT32 ^T	B6 ^T (= ATCC 23308 ^T)	L. W. Moore
<i>A. vitis</i>	AV18 ^T	ATCC 49767 ^T	ATCC
<i>A. larrymoorei</i>	AF3.10 ^T	ATCC 51759 ^T	This study
<i>A. larrymoorei</i>	AF3.44	—	This study
<i>A. larrymoorei</i>	AF1.72	—	This study

* ATCC, American Type Culture Collection (Manassas, VA, USA); L. W. Moore, Oregon State University (Corvallis, OR, USA).

Table 2. Comparison of DNA-reassociation values for *Agrobacterium* strains

Values represent percentage hybridization and are means of five separate hybridization measurements. Standard deviations are given in parentheses.

Strain used as probe	AF3.10 ^T	AF3.44	AF1.72	AT32 ^T	AV18 ^T	ARU2 ^T	AR13 ^T
AF3.10 ^T	100 (0)	89 (20)	100 (28)	36 (5)	33 (3)	37 (6)	37 (6)
AT32 ^T	58 (13)	NT	NT	100 (0)	49 (15)	57 (18)	54 (13)
AV18 ^T	49 (17)	NT	NT	43 (22)	100 (0)	48 (16)	52 (20)
ARU2 ^T	33 (10)	NT	NT	34 (10)	36 (10)	100 (0)	35 (13)
AR13 ^T	36 (12)	NT	NT	49 (6)	45 (7)	42 (6)	100 (0)

NT, Not tested.

was fragmented by three passages through a French pressure cell under 110 316 kPa (16000 p.s.i.), resulting in DNA fragments of ca. 0.5 kb. The DNA was heat-denatured and was either used to coat microtitre plates (MicroFluor type B; Dynatech Laboratory) or biotinylated to be used as a probe. Each microtitre well was coated with 3 µg fragmented, denatured DNA. The probe contained 20–50 ng DNA labelled with Photoprobe biotin (Vector Laboratories) according to the manufacturer's protocol. Hybridization was carried out at 52 °C for 12 h. DNA reassociation ratios were determined fluorometrically (7630 Microplate Fluorometer; Cambridge Technology) 1 h after the binding of the β -galactosidase avidin D (Vector Laboratories) and addition of the 4-methylumbelliferyl β -D-galactoside (Sigma) substrate.

The ability of the fig strains to oxidize 95 substrates from the BIOLOG GN MicroPlate was compared with that of strains of *Agrobacterium* species. Inoculation, incubation and analysis were performed as specified by the manufacturer.

The *Agrobacterium* strains isolated from *F. benjamina* had 89% or greater homology with each other but less than 60% with the other *Agrobacterium* species (Table 2). When DNA from the other *Agrobacterium* species was used as the probe, the level of homology with DNA from the fig bacterium was less than 60%. The differences between the *Agrobacterium* strains isolated

from *F. benjamina* and the type strains of the four other telluric *Agrobacterium* species were corroborated by *rrs* sequence analysis (Bouzar *et al.*, 1995), in which AF3.10^T had high rates of nucleotide substitution when compared with the type strains of the other four species. Based on DNA homology data and sequence analysis of the *rrs* (Bouzar *et al.*, 1995), the tumorigenic *Agrobacterium* strains isolated from aerial tumours of *F. benjamina* represent members of a novel species. The name *Agrobacterium larrymoorei* is proposed for this novel species after the late Larry W. Moore, a plant pathologist who was instrumental in understanding the ecology and diversity of agrobacteria. In this instance, combining the first and family names to create the specific epithet is necessary because Moore is a very common name.

The BIOLOG GN MicroPlate system was useful for distinguishing the *Agrobacterium* species (Bouzar *et al.*, 1993) and was used to characterize strains of *A. larrymoorei*. Strains of *A. larrymoorei* have metabolic activity on the carbon substrates *N*-acetyl D-glucosamine, adonitol, L-arabinose, D-arabitol, cellobiose, D-fructose, L-fucose, D-galactose, gentiobiose, α -D-glucose, *m*-inositol, lactulose, maltose, D-mannitol, D-mannose, D-melibiose, D-psicose, L-rhamnose, sucrose, D-trehalose, furanose, methyl pyruvate, mono-methyl succinate, DL-lactic acid, quinic acid, D-saccharic acid, succinic acid, bromosuccinic acid, L-alanine, L-alanyl-glycine, L-asparagine, L-aspartic acid, L-glutamic acid,

Fig. 1. Transmission micrograph of shadowed figure showing the presence of peritrichous flagella on a cell of *Agrobacterium larrymoorei*.

L-histidine, L-ornithine, L-proline, L-serine, L-threonine, glycerol, glucose 1-phosphate and glucose 6-phosphate. None of the strains had metabolic activity on the carbon substrates α -cyclodextrin, Tween 40, Tween 80, *N*-acetyl D-galactosamine, *i*-erythritol, α -lactose, D-raffinose, acetic acid, *cis*-aconitic acid, citric acid, D-glucosaminic acid, D-glucuronic acid, β -hydroxybutyric acid, γ -hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α -ketoglutaric acid, α -ketovaleric acid, malonic acid, sebamic acid, succinamic acid, glucuronamide, alaninamide, D-alanine, hydroxy-L-proline, L-leucine, L-phenylalanine, L-pyrogutamic acid, D-serine, DL-carnitine, γ -aminobutyric acid, urocanic acid, inosine, thymidine, phenylethylamine, 2-aminoethanol, 2,3-butanediol or DL- α -glycerol phosphate. *A. larrymoorei* was the only *Agrobacterium* species unable to metabolize *cis*-aconitic acid.

Description of *Agrobacterium larrymoorei* sp. nov.

Agrobacterium larrymoorei (lar.ry.moore'i. M.L. gen. *larrymoorei* of Larry Moore, renowned plant pathologist who spent his career studying the genus *Agrobacterium*).

The bacterium is a Gram-negative rod and a strict aerobe. Cells are motile with peritrichous flagella (Fig. 1). Cell and colony morphology and metabolic activity are as described for the genus (Sawada *et al.*, 1993). However, colonies growing on Roy-Sasser medium

(Moore *et al.*, 2001) are cream-white with a red centre, ca. 1 mm in diameter, circular, convex and glossy. The reaction of strains in litmus milk is alkaline with proteolysis (Bouzar *et al.*, 1995). Alkali is produced from tartaric acid, but not from malonic acid (Bouzar *et al.*, 1995). Strains do not produce 3-ketolactose from lactose, do not utilize citrate and do not grow at 35 °C (Bouzar *et al.*, 1995). The ferric ammonium citrate test is negative (Bouzar *et al.*, 1995). Strains produce acid from mannitol and grow on 2% NaCl (Bouzar *et al.*, 1995). Strains can be differentiated from those of one or more of the other four *Agrobacterium* species by their ability to metabolize the carbon substrates *N*-acetyl D-glucosamine, gentiobiose, *m*-inositol, lactulose, mono-methyl succinate, DL-lactic acid, D-saccharic acid, glucose 1-phosphate and glucose 6-phosphate and by their inability to metabolize Tween 40, Tween 80, *i*-erythritol, α -lactose, D-raffinose, acetic acid, citric acid, D-glucosaminic acid, malonic acid, succinamic acid, alaninamide, hydroxy-L-proline and L-pyrogutamic acid. A key characteristic that differentiates this species from other agrobacteria is its inability to metabolize *cis*-aconitic acid. The type strain of *A. larrymoorei* is AF3.10^T (= ATCC 51759^T = CFBP 5473^T = NCPPB 4096^T).

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