

Rhizobium ecuadorensis sp. nov., an indigenous N₂-fixing symbiont of the Ecuadorian common bean (*Phaseolus vulgaris* L.) genetic pool

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There are two major centres of genetic diversification of common bean (*Phaseolus vulgaris* L.), the Mesoamerican and the Andean, and the legume is capable of establishing nitrogen-fixing symbioses with several rhizobia; *Rhizobium etli* seems to be the dominant species in both centres. Another genetic pool of common bean, in Peru and Ecuador, is receiving increasing attention, and studies of microsymbionts from the region can help to increase our knowledge about coevolution of this symbiosis. We have previously reported several putative new lineages from this region and here present data indicating that strains belonging to one of them, PEL4, represent a novel species. Based on 16S rRNA gene sequence phylogeny, PEL4 strains are positioned in the *Rhizobium phaseoli/R. etli/Rhizobium leguminosarum* clade, but show unique properties in several morphological, physiological and biochemical analyses, as well as in BOX-PCR profiles (<75 % similarity with related species). PEL4 strains also differed from related species based on multilocus sequence analysis of three housekeeping genes (*glnII*, *gyrB* and *recA*). Nucleotide identities of the three concatenated genes between PEL4 strains and related species ranged from 91.8 to 94.2 %, being highest with *Rhizobium fabae*. DNA–DNA hybridization (<47 % DNA relatedness) and average nucleotide identity values of the whole genomes (<90.2 %) also supported the novel species status. The PEL4 strains were effective in nodulating and fixing N₂ with common beans. The data supported the view that PEL4 strains represent a novel species, *Rhizobium ecuadorensis* sp. nov. The type strain is CNPSo 671^T (=UMR 1450^T=PIMAMPIRS I 5^T=LMG 27578^T).

Common bean (*Phaseolus vulgaris* L.) is by far the most important legume cropped for human consumption worldwide (CGIAR, 2012). This legume is considered native to

the Americas, and domestication is thought to have taken place in two major centres of genetic diversification: the Mesoamerican centre or northern group (from Mexico to the northern region of South America – Colombia, Ecuador and north of Peru) and the Andean centre or southern group (from southern Peru to north-west Argentina) (Kaplan, 1965, 1981; Debouck, 1986; Debouck *et al.*, 1993; Rodiño *et al.*, 2010). Other centres of genetic diversification have also been suggested, in Colombia (Gepts,

Abbreviations: ANI, average nucleotide identity; DDH, DNA–DNA hybridization; MLSA, multilocus sequence analysis; PEL, *Rhizobium phaseoli/R. etli/Rhizobium leguminosarum*

Two supplementary figures and two supplementary tables are available with the online Supplementary Material.

1990; Kami *et al.*, 1995) and in Peru–Ecuador (Debouck *et al.*, 1993), the latter characterized by a specific storage seed protein, phaseolin type I (Kami *et al.*, 1995). Indeed, a hypothesis has been raised supporting the northern Peru–Ecuador region as the ancestral population of *P. vulgaris* (Kami *et al.*, 1995; Freyre *et al.*, 1996). Although recent analyses of nucleotide diversity have clearly indicated a Mesoamerican origin for common bean (Bitocchi *et al.*, 2013), with the Oaxaca valley in Mesoamerica and southern Bolivia and northern Argentina in South America as centres of domestication (Bitocchi *et al.*, 2013), the gene pool of Peru–Ecuador is unique in many aspects (Bitocchi *et al.*, 2012) and deserves further attention.

An important biological feature of common bean is its ability to interact with nitrogen-fixing rhizobia to obtain nitrogen for growth, with relevant economic, social and environmental impacts. Intriguingly, the legume is well known for its promiscuity in associating with a variety of species of the genus *Rhizobium*, establishing both effective (*Rhizobium leguminosarum* sv. *phaseoli*, *R. phaseoli*, *R. tropici*, *R. etli*, *R. leucaenae*, *R. giardinii* sv. *phaseoli*, *R. gallicum*, *R. lusitanum*, *R. pisi*, *R. freirei*, *R. mesoamericanum*, *R. paranaense*) and ineffective (*R. giardinii* sv. *giardinii* and *R. miluonense*) symbioses (Dall’Agnol *et al.*, 2014). However, there are relatively few studies regarding common bean–rhizobia coevolution in centres of origin/domestication of the legume. In Mexico, *R. etli* has been recognized as the dominant species (Segovia *et al.*, 1993), although other related lineages may also be important (López-Guerrero *et al.*, 2012). In north-west Argentina, *R. etli* has also been reported as the dominant species (Aguilar *et al.*, 1998, 2004). Therefore, *R. etli*-related species seem dominant in the centres of origin/domestication of common bean. In one study performed by our group with strains from northern and central

Ecuador (Bernal and Graham, 2001), although 50 % of the strains were positioned in the *R. phaseoli*/*R. etli*/*R. leguminosarum* (PEL) clade, there were clearly new lineages (Ribeiro *et al.*, 2013). By using a polyphasic approach, we now describe a novel species of the genus *Rhizobium*, comprising strains considered as lineage PEL4 in our previous study (Ribeiro *et al.*, 2013), and encompassing strains that seem dominant in Ecuador.

The six strains used in this study were isolated from common bean in different sites of Ecuador (CNPSo 670, 671^T, 672, 676 and 683) and one from Mexico (CNPSo 659) (Table 1). Strains from Ecuador were isolated by Dr Peter H. Graham of the University of Minnesota, USA, and are deposited at the Diazotrophic and Plant Growth Promoting Bacteria Culture Collection of Embrapa Soja (WFCC Collection no. 1213, WDCM Collection no. 1054), located in Londrina, State of Paraná, Brazil, and at the Center for Genomic Sciences Culture Collection (Cuernavaca, Mexico). Type strains of other species used in this study are listed in Table 1. Unless otherwise indicated, strains from this study were grown on yeast extract-mannitol agar (YMA) medium at 28 °C (Vincent, 1970). Stock cultures were maintained on YMA at 4 °C, while long-term preservation was performed in 30 % glycerol at –80 and –150 °C, or by lyophilization.

In a previous study based on 16S rRNA gene sequence and multilocus sequence analysis (MLSA) phylogenies, strains CNPSo 670, 671^T, 672, 676, 683 and 659 were inconsistently positioned in the PEL clade, which also includes *R. fabae* and *R. pisi* (Ribeiro *et al.*, 2013). To further characterize these strains, fingerprinting analysis was performed by BOX-PCR as described by Kaschuk *et al.* (2006). The profiles were compared with five reference strains of the PEL clade. Clusters were obtained with the BioNumerics program, v.7.1 (Applied Mathematics). We used

Table 1. Strains used in this study

Species/strain name	Other strain nomenclature	Geographical origin	Supplied by
CNPSo 670	UMR 1449, PIMAMPIRS I 4	San Jose, Imbaburra, Ecuador	University of Minnesota, USA
CNPSo 671 ^T	UMR 1450 ^T , PIMAMPIRS I 5 ^T	Pimampiro, Imbaburra, Ecuador	University of Minnesota, USA
CNPSo 672	UMR 1452, Los Olivos I 7	La Olivos, Imbaburra, Ecuador	University of Minnesota, USA
CNPSo 676	UMR 1462, Salsipuedes Ch 5	Salsipuedes, Chimborazo, Ecuador	University of Minnesota, USA
CNPSo 683	UMR 1490, Salapa L17	Salapa, Loja, Ecuador	University of Minnesota, USA
CNPSo 659	UMR 1271,023-85EF	Mexico	University of Minnesota, USA
<i>R. phaseoli</i> ATCC 14482 ^T	CNPSo 2057 ^T , 316c15 ^T , DSM 30137 ^T , IAM 12612 ^T , LMG 8819 ^T , 316c15 ^T		Centro de Ciencias Genómicas, Mexico
<i>R. fabae</i> CCBAU 33202 ^T	CNPSo 2059 ^T , CCBAU 33202 ^T , LMG 23997 ^T	Nanchang, Jiangxi, China	Centro de Ciencias Genómicas Mexico
<i>R. pisi</i> DSM 30132 ^T	CNPSo 2058 ^T	Rochelle, IL, USA	Centro de Ciencias Genómicas, Mexico
<i>R. etli</i> CFN 42 ^T	CNPSo 140 ^T , USDA 9032 ^T , ATCC 51251 ^T , DSM 11541 ^T , CNPSo 140 ^T	Mexico	Centro de Ciencias Genómicas, Mexico
<i>R. leguminosarum</i> USDA 2370 ^T	CNPSo 134 ^T , 3HOq18 ^T , ATCC 10004 ^T , LMG 11200 ^T , NCIMB 11478 ^T , SEMIA 320 ^T , ATCC 10004 ^T	Rochelle, IL, USA	USDA, Beltsville, USA

the UPGMA algorithm and the Jaccard coefficient with 2 % tolerance. Despite the high similarity found by BOX-PCR, it was possible to distinguish the PEL4 lineage from other reference strains. PEL4 strains were grouped with a similarity higher than 90 % (Fig. S1, available in the online Supplementary Material). The closest reference strain was *R. leguminosarum* USDA 2370^T, at 75 % similarity, whereas the other reference strains showed similarities lower than 70 % with the PEL4 lineage.

Two phylogenetic trees (16S rRNA and MLSA) were obtained for all described species of the genus *Rhizobium* of the PEL clade, including the six PEL4 strains and selected related taxa: *R. phaseoli* ATCC 14482^T, *R. fabae* CCBAU 33202^T, *R. pisi* DSM 30132^T, *R. etli* CFN 42^T, *R. leguminosarum* sv. *viciae* USDA 2370^T (Table 1), *R. miluonense* CCBAU 41251^T (=CNPSO 2056^T), *R. tropici* CIAT 899^T (=CNPSO 142^T), *R. multihospitium* CCBAU 83401^T (=CNPSO 2054), *R. hainanense* I66^T, *R. pseudoryzae* J3-A127^T, *R. pusense* NRCPB10^T, *R. lusitanum* P1-7^T (=CNPSO 2055), *R. rhizogenes* ATCC 11325^T, *R. leucaenae* CFN 299^T (=CNPSO 141), *R. gallicum* R602^T (=CNPSO 172), *R. radiobacter* C58, *R. galegae* LMG 6214^T (=CNPSO 2061) and *R. giardinii* H152^T (=CNPSO 172). *Bradyrhizobium diazoefficiens* USDA 110^T (=CNPSO 46^T) was used as the outgroup. 16S rRNA and housekeeping *glnII*, *gyrB* and *recA* gene sequences were retrieved from the GenBank database (accession numbers are given on the trees). Alignments and phylogenies were obtained with MEGA version 6.0 (Tamura *et al.*, 2013), using maximum-likelihood (Felsenstein, 1981), as suggested by Tindall *et al.* (2010), as well as by the neighbour-joining (Saitou & Nei, 1987) algorithm and K2P distances (Kimura, 1980). Statistical support for the trees was assessed by bootstrapping (Felsenstein, 1985) with 1000 replicates.

As observed by us previously (Ribeiro *et al.*, 2013), it was not possible to determine the correct phylogenetic position of the PEL4 strains by using the 16S rRNA gene (Fig. 1). By contrast, MLSA has been used with success to define *Rhizobium* species (e.g. Ribeiro *et al.*, 2009, 2012, 2013; Dall'Agnol *et al.*, 2013, 2014). In this study, MLSA with three concatenated housekeeping genes (*glnII*, *gyrB* and *recA*) allowed the clear separation of PEL4 strains to a single and consistent group with 100 % bootstrap support (Fig. 2). Note that MLSA with three housekeeping genes has been used previously as support for the description of novel rhizobial species (e.g. Delamuta *et al.*, 2013; Dall'Agnol *et al.*, 2014). A phylogenetic tree built with two housekeeping genes, *glnII* and *recA*, also supported the distinctiveness of this new lineage in relation to other recently proposed *Rhizobium* species of the PEL clade for which no *gyrB* gene sequences were available (Fig. S2).

Nucleotide identities of the 16S rRNA and housekeeping genes are given in Table S1, and PEL4 strains were compared both with PEL4 clade strains and with strains of other *Rhizobium* species. For the 16S rRNA gene, nucleotide identity values within PEL4 strains ranged from 99.6

to 100 %, and from 99.3 to 100 % for the three concatenated housekeeping genes. When PEL4 strains were compared with five species of the PEL clade, identities of the 16S rRNA gene ranged from 99 to 100 %, being highest (100 %) with *R. fabae* CCBAU 33202^T and *R. pisi* DSM 30132^T. For the concatenated housekeeping genes, values ranged from 91.8 to 94.2 %, being highest with *R. fabae* CCBAU 33202^T (Table S1).

DNA–DNA hybridization (DDH) was conducted by a filter hybridization methodology (Martínez-Romero *et al.*, 1991). Three strains (CNPSO 670, 671^T and 676), selected to represent the PEL4 group, were compared with five type strains of the closest neighbours within the PEL clade and also with the type strains of *R. tropici* and *R. leucaenae*, important symbionts of common bean. Tests were performed in two experiments, each one duplicated, with the genome of strain CNPSO 671^T used as the basis for hybridization, and the data presented are weighted averages of the two experiments (Table 2). When strain CNPSO 671^T was compared with CNPSO 670 and CNPSO 676, strains of the same species, the level of DNA–DNA relatedness was 87 and 71 %, respectively (Table 2). When the same strain was compared with other species of the genus *Rhizobium*, DDH values were lower than 47 %, the highest value being obtained with *R. phaseoli* ATCC 14482^T.

Average nucleotide identity (ANI) of whole genomes represents a useful alternative to DDH to estimate genome relatedness. Initially, Konstantinidis *et al.* (2006) proposed values of 96 % for housekeeping genes would correspond to 70 % DNA–DNA hybridization. By analysing more genomes, Richter & Rosselló-Móra (2009) suggested ANI values of 95–96 %, a range that was confirmed by Kim *et al.* (2014) based on the analysis of 6787 genomes. There are not many representative genomes of the PEL clade so we have used the genome sequence of strain CNPSO 671^T (sequenced for this study, SUB983818), and the genomes of *R. etli* CFN 42^T and *R. phaseoli* CIAT 652, available at the NCBI. ANI values were obtained using the program JSpecies (Richter & Rosselló-Móra, 2009) and the MUMmer system for sequence alignment. Strain CNPSO 671^T had ANI values of 89.2 and 90.2 % with the type strains of *R. etli* and *R. phaseoli*, respectively, values below the species circumscription threshold.

The fatty acid profile of strain CNPSO 671^T was determined as described by Delamuta *et al.* (2013), using the MIDI Sherlock Microbial Identification System with the TSBA6 database after growth on YMA for 5 days. The results, shown in Table S2, indicate summed feature 8 (C_{18:1} ω7c/ω9t/ω12t, C_{18:1} ω7c/ω9c/ω12t) as the major fatty acids, representing 52.3 %, in the range reported for other *Rhizobium* species (Tighe *et al.*, 2000). The profile of strain CNPSO 671^T differed from those of *R. etli* and *R. leguminosarum* mainly in C_{18:1} ω7c 11-methyl, C_{18:1} ω9c and C_{19:0} cyclo ω8c.

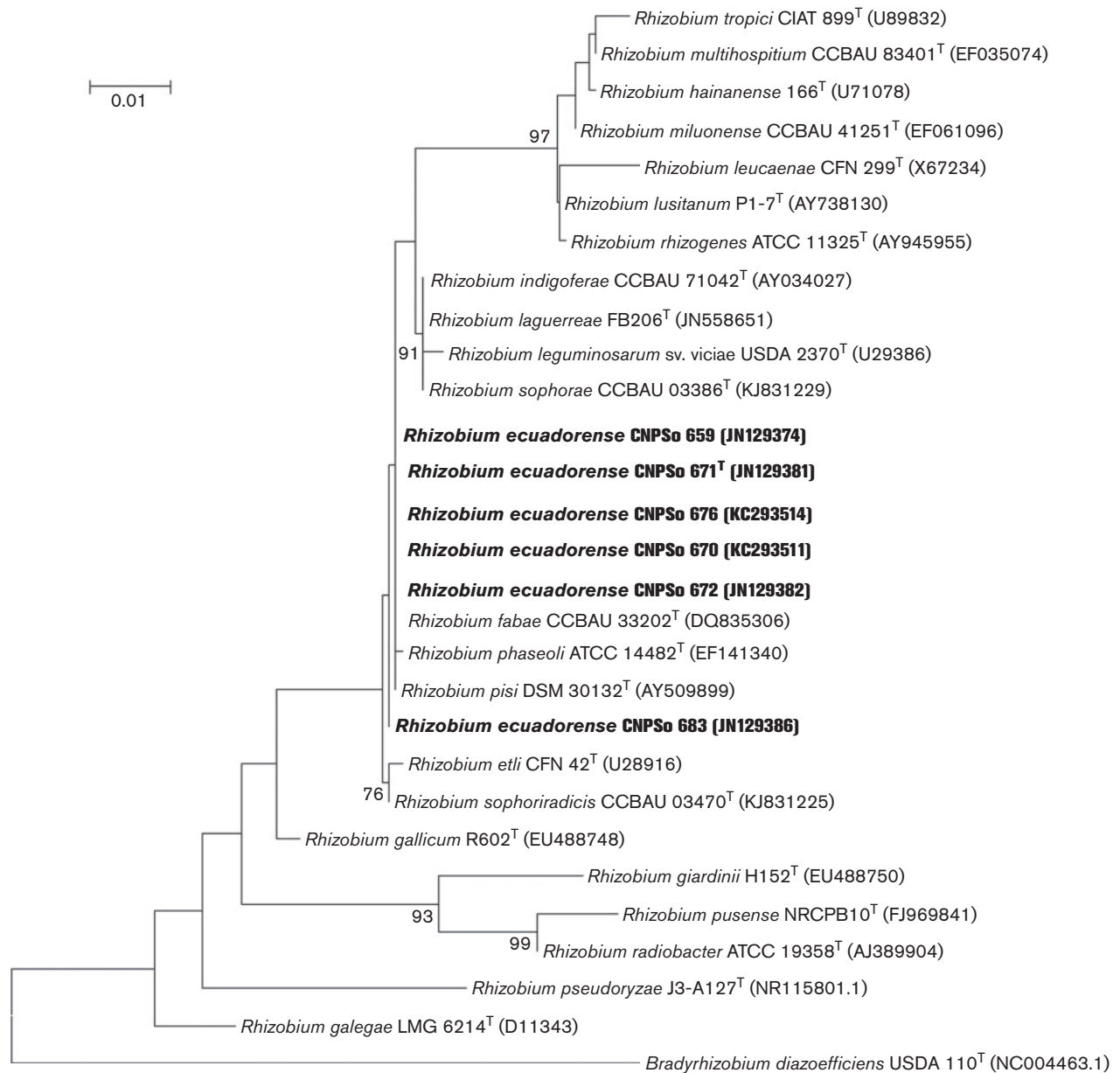


Fig. 1. Maximum-likelihood tree based on 16S rRNA gene sequences (1235 nt) of PEL4 strains (shown in bold) and type/reference strains. Bootstrap support values of $\geq 70\%$ are shown at nodes. GenBank accession numbers are provided in parentheses. Bar, 1 substitution per 100 nt positions.

To determine the DNA G+C content of strain CNPSO 671^T, genome contigs were concatenated and the proportion of G+C bases was calculated with BioEdit (Hall, 1999). The CNPSO 671^T genome had a G+C content of 61.07 mol%.

Evaluation of symbiotic capacity was performed in Leonard jars under sterile conditions. PEL4 strains were able to establish effective symbiosis with common bean of both Mesoamerican (cultivar Diamante Negro) and Andean

(cultivar Jalo Precoce) regions, but not with *Leucaena leucocephala* (data not shown).

Phenotypic tests and their reproducibility have been criticized (Ormeño-Orrillo & Martínez-Romero, 2013), mainly due to different results obtained in different laboratories and because the properties are not sufficient to identify species. However, they are important for strain characterization and are still required for rhizobial species definition; therefore, we determined several phenotypic

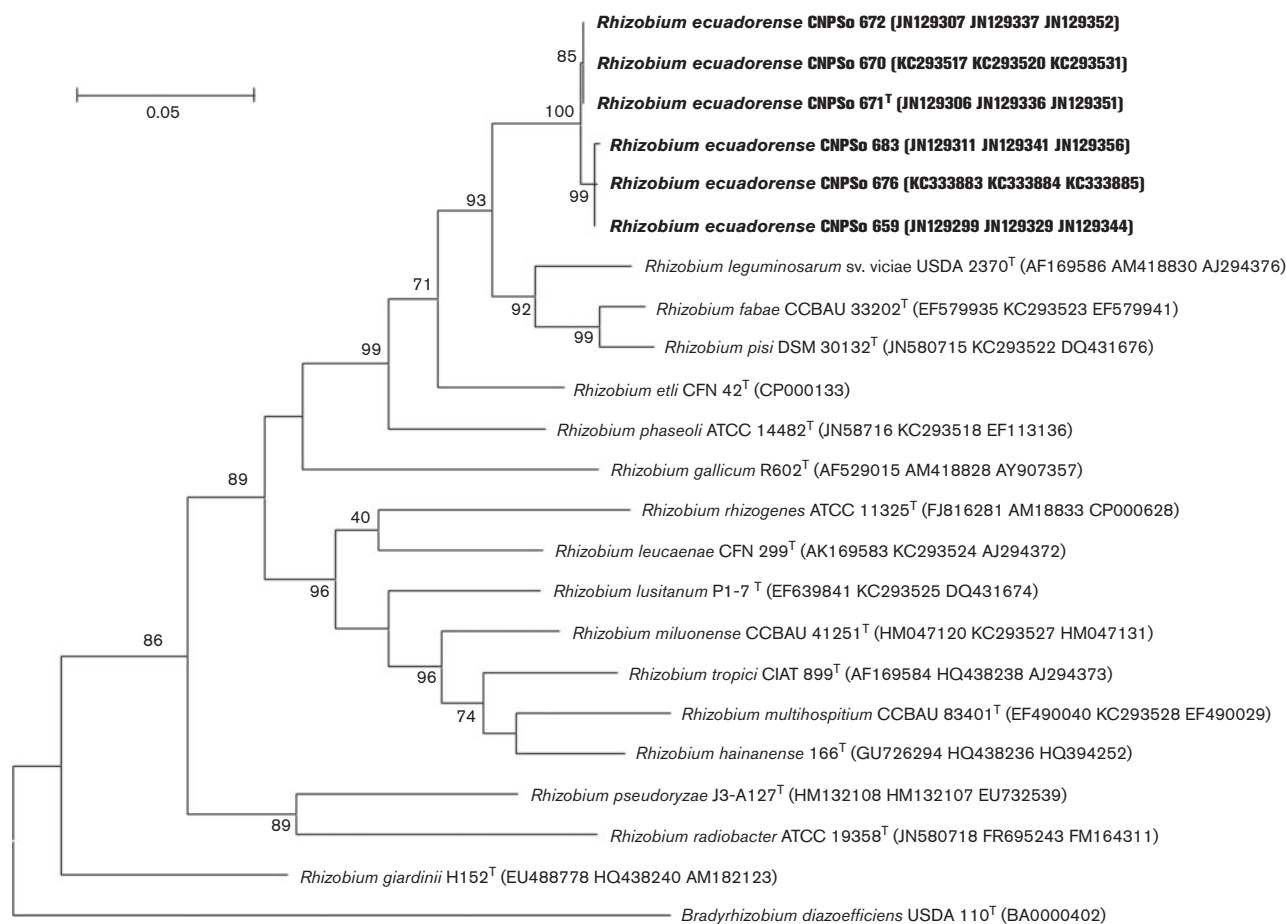


Fig. 2. Maximum-likelihood phylogenetic tree based on a concatenated alignment of *glnII* (450 nt), *gyrB* (583 nt) and *recA* (269 nt) gene sequences of PEL4 strains (shown in bold) and type/reference strains. Bootstrap support values of ≥ 70 % are shown at nodes. GenBank accession numbers are provided in parentheses. Bar, 5 substitutions per 100 nt positions.

Table 2. DDH between strain CNPSo 671^T and related strains

Values represent the weighted averages of two experiments, each with two replicates.

Strain	DDH (%)
CNPSo 671^T	100
CNPSo 670	87.95
CNPSo 676	71.05
<i>R. etli</i> CFN 42 ^T	31.34
<i>R. phaseoli</i> ATCC 14482 ^T	47.42
<i>R. leguminosarum</i> USDA 2370 ^T	34.03
<i>R. pisi</i> DSM 30132 ^T	39.16
<i>R. fabae</i> CCBAU 33202 ^T	28.85
<i>R. tropici</i> CIAT 899 ^T	14.92
<i>R. leucaenae</i> CFN 299 ^T	15.45

characteristics for the PEL4 group, in comparison with closely related reference strains. Unless indicated otherwise, all tests were performed at 28 °C. Colony morphology, acid production and tolerance to 1 % NaCl were determined on YMA; growth was also evaluated in Luria–Bertani (LB) medium, at different pH (pH 4.0 and 6.8) and temperatures (28, 37 and 40 °C) in liquid YM medium, and in PY medium lacking Ca, determined as described by Hungria *et al.* (2001). The enzymic degradation of urea was also evaluated using YMA medium supplemented with 2 % urea and in the presence of phenol red indicator. Tolerance to some antibiotics was evaluated using the disc diffusion method on YMA plates with the following antibiotics: bacitracin, chloramphenicol, cefuroxime, erythromycin, streptomycin, nalidixic acid, neomycin and tetracycline, and the concentrations evaluated are shown in Table 3. Carbon-source utilization was determined using the API 50CH kit (bioMérieux), according to the

Table 3. Distinctive phenotypic features of PEL4 strains and phylogenetically related *Rhizobium* species

Data represent the means of three biological replicates. Strains: 1, CNPSO 670; 2, CNPSO 671^T; 3, CNPSO 672; 4, CNPSO 676; 5, CNPSO 683; 6, CNPSO 659; 7, *R. leguminosarum* sv. *viciae* USDA 2370^T; 8, *R. fabae* CCBAU 33202^T; 9, *R. pisi* DSM 30132^T; 10, *R. etli* CFN 42^T; 11, *R. phaseoli* ATCC 14482^T. Growth was positive for all strains at pH 6.8 and 28 °C, but not at pH 4.0, 37 °C or 40 °C. None of the strains was able to grow in LB medium of PY medium lacking Ca. All strains were resistant to bacitracin (0.04 U) and sensitive to cefuroxime (30 µg). All were positive for D-arabinose, L-arabinose, D-manitol, D-ribose, L-xylose, aesculin and D-fucose as carbon sources, weakly positive for salicin, cellobiose, lactose, raffinose, gentiobiose and D-lyxose, but negative for inulin, melezitose, starch, potassium 2-ketogluconate and potassium 5-ketogluconate. +, Positive; w, weakly positive; -, negative.

	1	2	3	4	5	6	7	8	9	10	11
Growth with:*											
1 % NaCl	-	-	-	-	-	-	-	+	-	-	+
2 % Urea	-	-	-	-	w	-	-	w	-	-	-
Antibiotic tolerance (µg)											
Chloramphenicol (30)	-	-	w	-	-	-	w	+	-	-	-
Erythromycin (15)	w	+	-	-	-	w	-	-	-	w	-
Streptomycin (10)								+			
Nalidixic acid (30)	-	-	+	-	-	-	+	+	+	-	+
Neomycin (30)	w	-	+	w	-	w	-	w	+	-	w
Tetracycline (30)	-	-	-	-	-	-	+	-	-	-	-
Carbohydrate utilization†											
Glycerol	+	+	+	+	+	+	w	w	+	+	+
Erythritol	-	-	-	-	-	-	-	w	w	+	-
D-Xylose	-	+	+	w	+	+	w	w	w	+	w
D-Adonitol	-	-	-	w	w	w	w	w	w	w	w
Methyl β-D-xylopyranoside	+	w	+	w	+	w	w	w	w	w	w
D-Galactose	+	+	+	+	+	+	w	+	w	w	w
D-Glucose	w	w	w	w	w	w	w	+	w	w	w
D-Fructose	w	w	w	w	w	w	w	+	w	+	w
D-Mannose	+	w	w	w	w	w	w	+	w	+	w
L-Sorbose	-	-	-	-	-	-	w	w	w	-	-
L-Rhamnose	+	+	w	+	w	+	w	+	+	w	w
Dulcitol	-	-	-	-	-	-	w	w	w	w	-
Inositol	w	w	w	w	w	w	w	+	w	+	+
D-Sorbitol	-	w	w	w	w	-	w	w	w	w	w
Methyl α-D-mannopyranoside	-	-	-	-	-	-	w	w	w	-	-
Methyl α-D-glucopyranoside	w	w	w	w	w	w	w	w	+	w	w
N-Acetylglucosamine	w	w	w	w	w	w	-	-	+	+	-
Amygdalin	-	-	-	-	-	-	-	w	w	w	w
Arbutin	w	w	w	w	w	w	w	w	w	+	w
D-Maltose	w	w	w	w	w	w	w	+	w	+	w
Melibiose	w	+	w	w	w	w	w	w	w	w	w
Sucrose	w	+	w	w	w	w	w	w	w	+	w
Trehalose	w	+	w	w	w	w	w	w	w	+	w
Turanose	w	w	w	w	w	w	w	+	w	w	w
D-Tagatose	-	-	-	-	-	-	w	w	w	w	-
L-Fucose	w	+	+	+	w	w	w	+	+	+	w
D-Arabitol	+	+	+	w	w	+	w	+	w	w	w

*In liquid medium, growth (OD₆₀₀) was considered positive if ≥0.09, weak if in the range 0.04–0.09 and negative if ≤0.04.

†Carbon-source utilization was evaluated with the API 50CH kit (bioMérieux). +, Intense blue or yellow, depending on the carbon source; -, green; (weak, w) light blue or yellow.

manufacturer's instructions, using YM-minus-mannitol as the basal medium. All tests were performed in triplicate. In general, phenotypic results were uniform within the PEL4 lineage. The results of phenotypic tests that

differentiated the PEL4 lineage from other reference strains are shown in Table 3. The properties that were similar for all PEL4 strains are given in the species description below. One important feature was that, in contrast to the typical

symbionts of common bean in acid soils of Brazil (Martínez-Romero *et al.*, 1991; Hungria *et al.*, 2000; Ribeiro *et al.*, 2012; Dall'Agnol *et al.*, 2013, 2014) PELA4 strains are not tolerant to high temperatures and acidity (Table 3).

In conclusion, by means of a polyphasic approach comprising genotypic, phenotypic and phylogenetic data, the results indicate that strain CNPSo 671^T and the other PELA4 strains represent a novel species distinct from all described species in the genus *Rhizobium*, for which the name *Rhizobium ecuadorensis* sp. nov. is proposed.

Description of *Rhizobium ecuadorensis* sp. nov.

Rhizobium ecuadorensis (e.cu.a.dor.en'se. N.L. neut. adj. *ecuadorensis* of or belonging to Ecuador).

Cells are Gram-stain-negative, aerobic, non-spore-forming rods. Colonies on YMA medium are circular, opaque, with moderate to abundant production of mucus and usually 2–4 mm in diameter within 2–3 days of incubation at 28 °C. Strains acidify YMA medium after 3 days, do not grow at 37 or 40 °C or at pH 4; optimum growth occurs at pH 6.0–7.0 and at 25–28 °C. Strains do not grow in LB medium, PY medium lacking Ca or in the presence of 1 % NaCl. Tolerant to bacitracin (0.04 U) but sensitive to streptomycin (10 µg), tetracycline (30 µg) and cefuroxime (30 µg). In API tests, positive for D-arabinose, D-fucose, D-galactose, D-mannitol, D-ribose, aesculin, glycerol, L-arabinose and L-xylose as carbon sources, weakly positive for arbutin, cellobiose, D-fructose, D-glucose, lactose, D-lyxose, maltose, raffinose, turanose, gentiobiose, inositol, methyl α-D-glucopyranoside, N-acetylglucosamine and salicin, but negative for starch, amygdalin, melezitose, D-tagatose, dulcitol, erythritol, inulin, L-sorbose, methyl α-D-mannopyranoside, potassium 2-ketogluconate and potassium 5-ketogluconate. Nodulates and fixes nitrogen with common bean (*Phaseolus vulgaris* L.).

The type strain is CNPSo 671^T (=UMR 1450^T=PIMAM-PIRS I 5^T=LMG 27578^T), isolated from an effective nodule of *Phaseolus vulgaris* L. in Ecuador. The DNA G+C content of the type strain is 61.07 mol%. CNPSo 670, 672, 676 and 683 are additional strains of the species.

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