Reclassification of *Arthrobacter viscosus* as *Rhizobium viscosum* comb. nov

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**Abstract**

The species *Arthrobacter viscosus* was isolated from soil from Guatemala and it was classified into the genus *Arthrobacter* on the basis of phenotypic traits. Nevertheless, the results of 16S rRNA gene analysis indicated that this species is a member of the genus *Rhizobium*, with *Rhizobium alamii* GBV016ᵀ and *Rhizobium mesosinicum* CCBAU 25010ᵀ as the most closely related species with 99.64 and 99.48 % similarity, respectively. The similarity values for the *recA* gene are 92.2 and 94.4 % with respect to *R. alamii* GBV016ᵀ and *R. mesosinicum* CCBAU 25010ᵀ, respectively, and those for the *atpD* gene are 92.9 and 98.7 %, respectively. Results of DNA–DNA hybridization analysis yield averages of 46 and 41 % relatedness with respect to the type strains of *R. alamii* and *R. mesosinicum*, respectively. Phenotypic characteristics also differed from those of the most closely related species of the genus *Rhizobium*. Therefore, based on the data obtained in this study, we propose to classify strain LMG 16473ᵀ as representing a novel species named *Rhizobium viscosum* comb. nov. (type strain LMG 16473ᵀ=CECT 908ᵀ).

The species *Arthrobacter viscosus* was isolated from soil from Guatemala and described by Gasdorff et al. [1] and later included in the Approved Lists of Skerman et al. [2]. The classification as a member of the genus *Arthrobacter* was based on the results of phenotypic tests but this species differed in important characteristics with respect to other species of this genus [1]. The type strain of the species *A. viscosus* NRRRL B-1973ᵀ was sent to LMG (LMG 16473ᵀ) and NCIMB (NCIMB 9729ᵀ) collections. From this last collection it was sent to CECT where is conserved under the accession number CECT 908ᵀ. LMG 16473ᵀ was included in the work of Heyman et al. [3] who obtained its 16S rRNA gene sequence and found that it was related to members of the genus *Rhizobium*. Other authors have also mentioned that this strain is misclassified as representing a member of the genus *Arthrobacter* because it presents the highest 16S rRNA gene similarity with respect to the members of the genus *Rhizobium* [4, 5], nevertheless, the reclassification of this strain has not been formally proposed. Therefore, the objective of this work was to revise the taxonomic status of the type strain of *A. viscosus* through a polyphasic approach which allows it to be reclassified as representing a member of the genus *Rhizobium* as *Rhizobium viscosum* comb. nov.

Amplification and sequencing of the complete 16S rRNA gene was carried out according to the methods of Rivas et al. [6] and that of the *recA* and *atpD* genes according to the methods of Gaunt et al. [7]. The obtained sequences were compared with those from GenBank using the BLASTN program [8] and the 16S rRNA gene sequences were also compared with those from the EzTaxon-e server [9]. Sequences were aligned using the ClustalX software [10]. The distances were calculated according to Kimura’s two-parameter model [11]. Phylogenetic trees were inferred using neighbour-joining (NJ) [12] and maximum likelihood (ML) [13] analyses. MEGA5 software [14] was used for all analyses.

The 16S rRNA gene sequences of LMG 16473ᵀ and CECT 908ᵀ obtained in this study were identical with each other and with that available in Genbank for LMG 16473ᵀ with the accession number AJ639832; which was then used in the phylogenetic analysis of this gene. This sequence was related to those of species of the genus *Rhizobium*, its closest relatives being *Rhizobium alamii* GBV016ᵀ and *Rhizobium mesosinicum* CCBAU 25010ᵀ with 99.64 and 99.48 % similarity, respectively, according to the results of EzTaxon-e database analysis. These three strains formed an independent cluster after the NJ and ML phylogenetic analyses of this gene (Fig. S1, available in the online Supplementary Material); in Fig. 1 only the species more closely related to LMG 16473ᵀ are included. The results of analysis of the housekeeping genes *recA* and *atpD*, which have been sequenced for most species

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Two supplementary figures are available with the online Supplementary Material.
of the genus *Rhizobium*, confirmed that the strains deposited in the LMG and CECT collections are the same (data not shown) and therefore only those of LMG 16473<sup>T</sup> were included in the phylogenetic analyses of the recA and atpD genes, the results of which indicated that LMG 16473<sup>T</sup>, *R. alamii* GBV016<sup>T</sup> and *R. mesosinicum* CCBAU 25010<sup>T</sup> formed an independent cluster (Fig. S2). The similarity values for the recA gene are 92.2 and 94.4 % with respect to *Rhizobium alamii* GBV016<sup>T</sup> and *R. mesosinicum* CCBAU 25010<sup>T</sup>, respectively and those for the atpD gene are 92.9 and 98.7 %, respectively. These values are in the range of those presented by several species of genus *Rhizobium* (Fig. S2) indicating that LMG 16473<sup>T</sup> does not represent a previously described species of this genus.

DNA–DNA hybridization experiments were carried out as reported previously [15, 16]. LMG 16473<sup>T</sup> showed averages of 46 % (reciprocal values 44/47) and 41 % (reciprocal values 39/42) DNA–DNA relatedness with respect to *R. alamii* LMG 24466<sup>T</sup> (GBV016<sup>T</sup>) and *R. mesosinicum* LMG 24135<sup>T</sup> (CCBAU 25010<sup>T</sup>), its closest relatives according to the housekeeping gene analysis. Since this percentage is below the 70 % threshold value of DNA–DNA similarity for definition of bacterial species [17], it is proposed that LMG 16473<sup>T</sup> should be assigned to a different species.

DNA for analysis of DNA base composition was prepared according to the protocol of Chun and Goodfellow [18]. The mol% G+C content of DNA was determined using the thermal denaturation method [19]. The DNA G+C content of LMG 16473<sup>T</sup> was 61.5 %, which is within the range reported for species of the genus *Rhizobium* [20].

The phenotypic characterization was performed using the tests and methodologies reported in the original description of *A. viscosus* [1] and completed with classical tests used in the characterization of species of the genus *Rhizobium* [21, 22] including the API ID32GN and API 20NE systems (with the addition of MgSO<sub>4</sub> up to a final concentration of 0.02 g l<sup>−1</sup>). The results were read after 7 days of incubation at 28 °C. Growth temperature range was determined by incubating cultures in yeast–mannitol agar (YMA) medium at 4, 15, 28, 37 and 45 °C. Growth pH range was determined in the same medium with final pH values of 4, 5, 6, 7, 8, 9 and 10. Salt tolerance was tested in the same medium containing 0.5, 1, 1.5, 2 and 2.5 % (w/v) NaCl. To test the natural antibiotic resistance, the disc diffusion method on YMA medium was used. The results were read after 3 days of incubation at 28 °C. The discs contained the following antibiotics: ampicillin (2 µg), erythromycin (2 µg), ciprofloxacin (5 µg), penicillin (11 IU), polymyxin (300 IU), cloxacillin (1 µg), oxytetracycline (30 µg), gentamycin (10 µg), cefuroxime (30 µg), or neomycin (5 µg), (Becton Dickinson, BBL). A growth inhibition halo further than 2 mm from the disc edge was considered as indicative of antibiotic sensitivity. The type strains of closely related species of the genus *Rhizobium*, *R. ali- mii* LMG 1473<sup>T</sup> (GBV016<sup>T</sup>) and *R. mesosinicum* LMG 24135<sup>T</sup> (CCBAU 25010<sup>T</sup>), were included in the phenotypic study as reference strains. Phenotypic characteristics of the novel species are reported below in the species description and the differences with respect to the most closely related species of the genus *Rhizobium* are recorded in Table 1.

The ability to nodulate legumes was included in the minimal standards for the description of species of the genus *Rhizobium* after the proposal of Graham et al. [23], who recommended *Macroptilium atropurpureum* for the nodulation assays. Therefore, we carried out nodulation tests as previously described, including *Rhizobium tropici* CIAT 899<sup>T</sup> as a positive control [24]. No nodules were observed in the roots of *M. atropurpureum* plants inoculated with LMG 16473<sup>T</sup> or CECT 908<sup>T</sup> five weeks after inoculation. PCR amplification of the nodC gene, which is part of the common nodulation genes nodABC involved in host specificity [25], was performed according to the method of Laguerre et al. [26] using *R. tropici* CIAT 899<sup>T</sup> as a positive control. The nodC gene amplification failed in LMG 16473<sup>T</sup> and

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**Fig. 1.** Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences (1424 positions) showing the relationships between *Rhizobium viscosum* LMG 16473<sup>T</sup> and other species of the genus *Rhizobium*. The significance of each branch is indicated by a bootstrap value (as a percentage) calculated for 1000 subsets (only values higher than 50 % are indicated). Bar, 1 substitution per 100 nucleotide positions. The nodes marked with filled circles were also obtained with the maximum likelihood algorithm.
CECT 908T whereas it was amplified in the positive control
R. tropici CIAT 899T (data not shown).

Therefore, on the basis of phenotypic and genotypic characteristics
we propose to reclassify the type strain of the species
A. viscosus as representing a novel species named
Rhizobium viscosum comb. nov.

**DESCRIPTION OF RHIZOBIUM VISCOSUM COMB. NOV.**

*Rhizobium viscosum* (vis.co’sum. L. neut. adj. viscosum,
because of its thread-forming, adherent colonies).


The description coincides with the original description of A.
viscosus [1] except for citrate assimilation, which is negative,
and growth at 37 °C, which is positive. Additional characteristics
from this study are as follows: Growth in the presence
of 1 % (w/v) NaCl is positive. Nitrate reduction is positive.
The hydrolysis of urea and aesculin is positive. The produc-
tion of α-arabinosidase, α and β-glucosidases, α and β-
galactosidases, β-glucosaminidase, β-galactosaminidase, α
and β-fucosidases, α and β-mannosidases, β-xilosidase, α-
rhamnosidase and alkaline phosphatase is positive. The pro-
duction of acid phosphatase, β-cellulobiase and β-maltosidase
is weak. The production of β-arabinosidase, galacturon-
idase, glucuronidase, lactosidase, α-maltosidase and α-xilo-
sidase is negative. The production of indole, arginine
dehydrodase and gelatinase is negative. The assimilation of
mannose, malate, L-rhamnose, N-acetyl-glucosamine, D-
ribose, inositol, sucrose, maltose, mannitol, glucose, salicin,
melibiose, L-fucose, L-sorbitose, L-arabinose, lactate, L-al-
nine, L-histidine and L-proline is positive. The assimilation
of itaconate, suberate, malonate, acetate, 2 and 5 keto-
gluconate, glycogen, 3-hydroxybenzoate, L-serine, propionate,
gluconate, caprate, adipate, phenylacetate, valerate, citrate,
3-hydroxybutyrate and 4-hydroxybenzoate is negative. Sen-
sitive to ciprofloxacin, oxitetracycline, cefuroxime, neomycin
and gentamycin. Resistant to erythromycin, penicillin,
ampicillin, polymyxin B and cloxacillin.

The type strain, LMG 16473T (=CECT 908T), was isolated
from soil from Guatemala. The DNA G+C content of
the type strain is 61.5 mol%.

**Table 1.** Differential characteristics of *R. viscosum* LMG 16473T
and related type strains

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<tbody>
<tr>
<td>Growth at/with:</td>
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<tr>
<td>pH 5</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>37 °C</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>0.5 % NaCl</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>β-l-Arabinosidase</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td>β-d-Mannosidase</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<td>α-l-Rhamnosidase</td>
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<tr>
<td>β-d-Xylosidase</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<td>Assimilation of (API 20NE):</td>
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<tr>
<td>Malate</td>
<td>–</td>
<td>+</td>
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<td>Gluconate</td>
<td>–</td>
<td>+</td>
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<td>Assimilation of (API 32GN):</td>
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<tr>
<td>4-Hydroxybenzoate</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>L-Proline</td>
<td>+</td>
<td>+</td>
<td>–</td>
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**Conflicts of interest**

The authors declare that there are no conflicts of interest.

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