Reclassification of *Agromonas oligotrophica* into the genus *Bradyrhizobium* as *Bradyrhizobium oligotrophicum* comb. nov.

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*Agromonas oligotrophica* JCM 1494ᵀ was isolated in Japan in 1983, and the name was validly published in 1985. Analysis of the 16S rRNA gene sequence showed that *Agromonas oligotrophica* LMG 10732ᵀ (=JCM 1494ᵀ) is located within the genus *Bradyrhizobium*, with *Bradyrhizobium denitrificans* LMG 8443ᵀ as its closest relative, showing 99.6% 16S rRNA gene sequence identity. However, *Agromonas oligotrophica* LMG 10732ᵀ and *Bradyrhizobium denitrificans* LMG 8443ᵀ can be distinguished by housekeeping gene sequence analysis, phenotypic characterization and DNA–DNA hybridization. *Agromonas oligotrophica* is also genotypically and phenotypically different from the remaining species of the genus *Bradyrhizobium*, and we therefore propose the reclassification of *Agromonas oligotrophica* into the genus *Bradyrhizobium* as *Bradyrhizobium oligotrophicum* comb. nov. (type strain LMG 10732ᵀ = JCM 1494ᵀ = ATCC 43045ᵀ).

The genus *Agromonas* was described by Ohta & Hattori (1983, 1985) and the genus currently accommodates a single species, *Agromonas oligotrophica*, isolated from rice paddy soils in Japan (Ohta & Hattori, 1983). The original type strain of this species, S58ᵀ, was deposited in the JCM culture collection as JCM 1494ᵀ and was later distributed to the NCIMB, ATCC and LMG culture collections with the accessions NCIB 12151ᵀ, ATCC 43045ᵀ and LMG 10732ᵀ. The phylogenetic closeness of this species to *Bradyrhizobium* species and to *Blastobacter denitrificans* was pointed out by Willems et al. (2001a), but no taxonomic decisions were taken to give a correct classification. More recently, the species *Blastobacter denitrificans* was reclassified in the genus *Bradyrhizobium* as *Bradyrhizobium denitrificans* (van Berkum et al., 2006, 2011). This has shown the need for the reclassification of *A. oligotrophica* in the genus *Bradyrhizobium*, since several strains isolated from legume nodules are closely related to this species, which can lead to confusion among researchers. This is the case for some photosynthetic strains that are almost equidistant from *A. oligotrophica* and *Bradyrhizobium denitrificans* (Fig. S1, available in IJSEM Online). This was not reported previously, because *A. oligotrophica* was not included in 16S rRNA gene phylogenetic analyses of *Bradyrhizobium*, since it did not belong to this genus (Molouba et al., 1999).

In the present work, after the analysis of several core gene sequences, we confirmed that *A. oligotrophica* belongs to the genus *Bradyrhizobium*, being closely related to *Bradyrhizobium denitrificans* (formerly *Blastobacter denitrificans*). These two species can be distinguished by core gene sequence analysis, phenotypic characterization and DNA–DNA hybridization.

The 16S rRNA gene sequence of *A. oligotrophica* LMG 10732ᵀ was obtained according to Rivas et al. (2007) and was compared with those from the EzTaxon database (Kim et al., 2012). Sequences of the recA and atpD genes were obtained as described by Gaunt et al. (2001) and the sequence of the glnII gene was obtained as described by Vinuesa et al. (2005a). These sequences were aligned with corresponding sequences from all *Bradyrhizobium* species

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, recA, atpD and glnII gene sequences of *Agromonas oligotrophica* LMG 10732ᵀ are respectively J0619230–J0619233.

A supplementary figure and a supplementary table are available with the online version of this paper.
using the CLUSTAL W program (Thompson et al., 1997). Distances were calculated according to Kimura’s two-parameter model (Kimura, 1980). Phylogenetic trees were inferred using neighbour-joining analysis (Saitou & Nei, 1987). MEGA 5.0 (Tamura et al., 2011) was used for all the analyses. Bootstrap analysis was based on 1000 resamplings.

Sequence analysis of the 16S rRNA gene showed that A. oligotrophica LMG 10732\textsuperscript{T} is located within the genus Bradyrhizobium, with Bradyrhizobium denitrificans LMG 8443\textsuperscript{T} as its closest relative (Fig. 1). The 16S rRNA gene sequence of A. oligotrophica LMG 10732\textsuperscript{T} was identical to that available for strain JCM 1494\textsuperscript{T}; however, the sequence deposited for the latter strain is short, and only LMG 10732\textsuperscript{T} is therefore included in the phylogenetic tree. The sequence identity between A. oligotrophica LMG 10732\textsuperscript{T} and Bradyrhizobium denitrificans LMG 8443\textsuperscript{T} was 99.6 % according to the EzTaxon database. The remaining type strains of the genus Bradyrhizobium presented less than 98.8 % 16S rRNA gene sequence identity. Despite the high identity found between the sequences of A. oligotrophica LMG 10732\textsuperscript{T} and Bradyrhizobium denitrificans LMG 8443\textsuperscript{T}, they may belong to different species, since values near 100 % are common among Bradyrhizobium species that present divergent housekeeping gene sequences and low DNA–DNA hybridization (Ramı́rez-Bahena et al., 2009; Chahboune et al., 2011).

We therefore analysed the sequences of the recA, atpD and glnII genes, since their analysis has been reported to allow the differentiation of Bradyrhizobium species with closely related 16S rRNA genes (Vinuesa et al., 2005a, b; Ramı́rez-Bahena et al., 2009; Rivas et al., 2009; Chahboune et al., 2011). Sequence analysis of these three genes showed that A. oligotrophica LMG 10732\textsuperscript{T}, although related to Bradyrhizobium denitrificans, formed a very divergent branch (Fig. 2). The identity between A. oligotrophica LMG 10732\textsuperscript{T} and Bradyrhizobium denitrificans LMG 8443\textsuperscript{T} was 91.9, 92.0 and 93.5 % for the recA, atpD and glnII genes, respectively. These values are lower than those found between some species of Bradyrhizobium with validly published names, such as Bradyrhizobium japonicum and Bradyrhizobium betae (>93.5 % sequence identity for the three genes), Bradyrhizobium pachyrhizi and Bradyrhizobium elkanii (>95 %) or Bradyrhizobium jicamae and Bradyrhizobium lablabi (>94 %), suggesting that strain LMG 10732\textsuperscript{T} belongs to a species different from Bradyrhizobium denitrificans.

DNA–DNA hybridization experiments were carried out using the method of Ezaki et al. (1989) following the recommendations of Willems et al. (2001b). A. oligotrophica LMG 10732\textsuperscript{T} and Bradyrhizobium denitrificans LMG 8443\textsuperscript{T} showed 46 % DNA–DNA relatedness. When we consider 70 % as the threshold value of DNA–DNA hybridization for definition of bacterial species (Wayne et al., 1987), strain LMG 10732\textsuperscript{T} belongs to a species different from Bradyrhizobium denitrificans.

DNA for analysis of DNA base composition was prepared according to Chun & Goodfellow (1995) and the DNA G+C content was determined using the thermal denaturation method (Mandel & Marmur, 1968). The DNA G+C content of A. oligotrophica LMG 10732\textsuperscript{T} was 65.1 mol%.

The phenotypic characterization recorded in the second edition of Bergey’s Manual (Kennedy, 2005) was completed using API 20NE and API ID32GN galleries that were inoculated according to the manufacturer’s instructions, and the results were recorded after 3 days of inoculation. Our results obtained by using these systems were coincident with those recorded by Kennedy (2005). According to these data, A. oligotrophica LMG 10732\textsuperscript{T} differed from Bradyrhizobium denitrificans LMG 8443\textsuperscript{T} in the type of flagellation, which is polar in the case of A. oligotrophica LMG 10732\textsuperscript{T} and subpolar in the case of Bradyrhizobium denitrificans LMG 8443\textsuperscript{T}, in acid production.

**Fig. 1.** Neighbour-joining phylogenetic tree based on nearly complete 16S rRNA gene sequences of Bradyrhizobium oligotrophicum comb. nov. LMG 10732\textsuperscript{T} and members of other species of the genus Bradyrhizobium. The significance of each branch is indicated by a bootstrap value calculated for 1000 subsets. Bar, 1 substitution per 100 nucleotide positions.
from glucose, denitrification and assimilation of methanol, which are negative for *A. oligotrophica* LMG 10732T, and in nitrogen fixation in culture, which was positive for *A. oligotrophica* LMG 10732T (Kennedy, 2005). Other phenotypic data obtained in this study and not reported by Kennedy (2005) are listed in the species description. According to these data, *A. oligotrophica* LMG 10732T and *Bradyrhizobium oligotrophicum* LMG 8443T also differed in assimilation of N-acetylglucosamine and sorbitol as carbon sources, which was negative for *A. oligotrophica* LMG 10732T. Phenotypic differences with respect to species of the genus *Bradyrhizobium* are recorded in Table S1.

Therefore, based on phenotypic and genotypic characteristics, we propose to reclassify *Agromonas oligotrophica* as *Bradyrhizobium oligotrophicum* comb. nov.

**Description of Bradyrhizobium oligotrophicum (Ohta and Hattori 1985) comb. nov.**

*Bradyrhizobium oligotrophicum* [o.li.goтро’phi.cum. Gr. adj. *oligos* little, small, few; N.L. neut. adj. *trophicum* (from Gr. neut. adj. *trophikon* nursing, tending or feeding; N.L. neut. adj. *oligotrophicum* oligotrophic, eating little nutrient].

Basonym: *Agromonas oligotrophica* Ohta and Hattori 1985. According to the original description of *A. oligotrophica* (Ohta & Hattori, 1983) and as reported by Kennedy (2005), the characteristics of this species are as follows. Bent, branched and budding cells, 0.6–1.0 μm. Motile by a polar flagellum. Several cells adhere to each other and form a rosette. Gram-negative. On dilute nutrient broth agar, colonies are punctiform, pulvinate, entire and colourless. Aerobic. Oligotrophic; i.e. growth can occur in a medium containing less than 1 mg of an organic carbon source g−1. Catalase- and oxidase-positive. Not proteolytic to casein and gelatin. Cellulose and starch are not hydrolysed. Atmospheric nitrogen is fixed under low O2 pressure. Growth at 37 °C is positive. Cellular fatty acids consist mainly of C18:1; small amounts of C16:0 and a 19-carbon unsaturated acid with a double bond and possibly with a side chain are found as minor components. Ubiquinone Q-10 is present. NaCl, KCl, Casamino acids, peptone and meat extract inhibit growth at 0.5–1.0%. Neither acid nor gas is produced from glucose. Assimilation of glucose, galactose, mannose, xylose, L-arabinose, acetic acid, lactic acid, gluconic acid, pyruvic acid, citric acid, 2-oxoglutaric acid, succinic acid, l-malic acid, ferulic acid, p-coumaric acid and p-anisic acid is positive. Assimilation of cellobiose, lactose, raffinose, benzoic acid and methanol is negative. Additional characteristics determined in this study using API 20NE and 32GN kits are as follows. In the API 20NE system, nitrate reduction and urease production are positive. Arginine dihydrolase is negative. Aesculin hydrolysis and β-galactosidase are weak. Assimilation of mannitol is positive. Assimilation of N-acetylgulcosamine, maltose, caprate and phenylacetate is negative. Assimilation of adipate is weak. In the API ID32GN system, assimilation of L-hamnosone, L-ribose, suberate, 2- and 5-ketogluconate, L-fucose, valerate, 3-hydroxybenzoate and 3-hydroxybutyrate is positive. Assimilation of N-acetylgulcosamine, inositol, succrose, itaconate, malonate, salicin, melibiose, sorbitol, caprate, 1-histidine, L-serine and L-proline is negative. Assimilation of propionate is weak.

The type strain, LMG 10732T (=JCM 1494T =ATCC 43045T), was isolated from a rice paddy soil in Japan. The DNA G+C content of the type strain is 65.1 mol%.

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References


