Bradyrhizobium canariense sp. nov., an acid-tolerant endosymbiont that nodulates endemic genistoid legumes (Papilionoideae: Genisteae) from the Canary Islands, along with *Bradyrhizobium japonicum* bv. *genistearum*, *Bradyrhizobium* genospecies alpha and *Bradyrhizobium* genospecies beta

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Highly diverse *Bradyrhizobium* strains nodulate genistoid legumes (brooms) in the Canary Islands, Morocco, Spain and the Americas. Phylogenetic analyses of ITS, *atpD*, *glnII* and *recA* sequences revealed that these isolates represent at least four distinct evolutionary lineages within the genus, namely *Bradyrhizobium japonicum* and three unnamed genospecies. DNA–DNA hybridization experiments confirmed that one of the latter represents a new taxonomic species for which the name *Bradyrhizobium canariense* is proposed. *B. canariense* populations experience homologous recombination at housekeeping loci, but are sexually isolated from sympatric *B. japonicum* bv. *genistearum* strains in soils of the Canary Islands. *B. canariense* strains are highly acid-tolerant, nodulate diverse legumes in the tribes Genisteae and Loteae, but not *Glycine* species, whereas acid-sensitive *B. japonicum* soybean isolates such as USDA 6^T and USDA 110 do not nodulate genistoid legumes. Based on host-range experiments and phylogenetic analyses of symbiotic *nifH* and *nodC* sequences, the biovarieties *genistearum* and *glycinearum* for the genistoid legume and soybean isolates, respectively, were proposed. *B. canariense* bv. *genistearum* strains display an overlapped host range with *B. japonicum* bv. *genistearum* isolates, both sharing monophyletic *nifH* and *nodC* alleles, possibly due to the lateral transfer of a conjugative chromosomal symbiotic island across species. *B. canariense* is the sister species of *B. japonicum*, as inferred from a maximum-likelihood *Bradyrhizobium* species phylogeny estimated from congruent *glnII*+*recA* sequence partitions, which resolves eight species clades. In addition to the currently described species, this phylogeny uncovered the novel *Bradyrhizobium* genospecies alpha and beta and the photosynthetic strains as independent evolutionary lineages. The type strain for *B. canariense* is BTA-1^T (=ATCC BAA-1002^T=LMG 22265^T=CFNE 1008^T).

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Abbreviations: ECGL, endemic Canarian genistoid legume; REP, repetitive extragenic palindromic sequence.

The GenBank/EMBL/DDBJ accession number for the *rrs* sequence of strain BC-C2 is AY577427; those for the ITS1 sequences are AY386703–AY386705, AY386707, AY386708, AY386712–AY386718, AY386721, AY386722, AY386734, AY599094 and AY599095.

Sequence accession numbers for new *Bradyrhizobium* sequences used and generated in this study, the figures discussed in the text and our final and concluding remarks are available as supplementary material in IJSEM Online.

In previous reports, we have shown that at least four *Bradyrhizobium* lineages nodulate endemic genistoid legumes such as *Adenocarpus*, *Chamaecytisus*, *Lupinus*, *Spartocytisus* and *Teline* species in soils of the Canary Islands (Fig. A, available as supplementary material in IJSEM Online). They are consistently distinguished by PCR-RFLPs of *rrs*+ITS amplicons (Jarabo-Lorenzo *et al.*, 2003; Vinuesa *et al.*, 1998, 1999), profiling of stable low-molecular-weight RNAs (Jarabo-Lorenzo *et al.*, 2000) and phylogenetic analyses of ITS (Fig. B, available as supplementary material in IJSEM Online), *atpD*, *glnII* and *recA* sequences using maximum-likelihood and Bayesian inference methods (Vinuesa *et al.*, 2005). We have shown that these lineages can be also recovered from the nodules of other genistoid legumes such as *Lupinus* spp. and from *Ornithopus* spp. (Papilionoideae: Loteae) growing in Africa, Europe and America (Jarabo-Lorenzo *et al.*, 2003; Vinuesa & Silva, 2004; Vinuesa *et al.*, 2005), using diverse complex media containing yeast-extract and mannitol such as YMA or 20E and following standard isolation procedures (León-Barrios *et al.*, 1991; Vinuesa *et al.*, 1998).

Bradyrhizobium japonicum is one of the lineages that nodulates endemic Canarian genistoid legumes (ECGLs) (Vinuesa *et al.*, 2005). The other three lineages represent unnamed genospecies that are clearly delineated in a well resolved species phylogeny based on combined *glnII*+*recA* sequences (see Fig. C, available as supplementary material in IJSEM Online).

Here we present evidence for the taxonomic distinctiveness (Stackebrandt *et al.*, 2002; Vandamme *et al.*, 1996) of one of these evolutionary lineages, for which we propose the name *Bradyrhizobium canariense*. This species can be unequivocally distinguished from the five *Bradyrhizobium* species currently described, namely *B. japonicum* (Jordan, 1982, 1984), *Bradyrhizobium elkanii* (Kuykendall *et al.*, 1992), *Bradyrhizobium liaoningense* (Xu *et al.*, 1995), *Bradyrhizobium yuanmingense* (Yao *et al.*, 2002) and *Bradyrhizobium betae* (Rivas *et al.*, 2004), by a combination of genotypic, physiological and ecological characteristics. *B. betae* was recently isolated from tumour-like root deformations of sugar beet (*Beta vulgaris*) in Northern Spain and has an unknown symbiotic status. It is possible that the four isolates used for the species description actually represent a single clone, since all presented the same ITS haplotype (Rivas *et al.*, 2004). *B. yuanmingense* was isolated from the root nodules of *Lespedeza* spp. growing in China, whereas the other three species were isolated from the nodules of soybean (*Glycine max*) in different continents (see Table A and figures provided as supplementary material in IJSEM Online).

B. canariense strains are grouped in highly supported monophyletic clusters in the gene trees inferred from a large number of ITS (Fig. B in IJSEM Online), *atpD*, *glnII* and *recA* sequences obtained from isolates of ECGLs and a diverse worldwide collection of *Bradyrhizobium* strains, including the type strains of all previously described species in the genus (Vinuesa *et al.*, 2005). Population genetics studies of Moroccan and Canarian *B. canariense* isolates (Vinuesa *et al.*, 2005) based on repetitive extragenic palindromic sequence (REP)-PCR genomic fingerprints, multilocus enzyme electrophoresis (MLEE) and multilocus sequence (*atpD*, *glnII*, *recA*) polymorphisms revealed: i) high levels of strain diversity across sampling sites; ii) significant levels of recombination, as assessed by linkage disequilibrium analyses of MLEE data, variance- and coalescent-based estimation methods of the population recombination parameter, and the reticulated evolutionary pattern exhibited by the ITS, *atpD*, *glnII* and *recA* sequence partitions; iii) lack of genetic differentiation between continental and insular populations; and iv) significant gene flow between them. From these findings it was inferred that migration and recombination are significant evolutionary forces that provide *B. canariense* with internal cohesiveness and shape its genetic population structure (Vinuesa & Silva, 2004; Vinuesa *et al.*, 2005). Furthermore, these population genetics studies revealed that there is no significant recombination between *B. canariense* strains and the other three sympatric evolutionary lineages recovered from the nodules of ECGLs, and that the genetic differentiation between these lineages is highly significant (Vinuesa *et al.*, 2005). This finding is remarkable since the four species have overlapped ecological niches and therefore the ecological opportunity for horizontal gene transfer. In conclusion, these studies demonstrated that *B. canariense* represents a *bona fide* evolutionary, phylogenetic and cohesive species (Mayr, 1970; Templeton, 1989; Ward, 1998; Wiley, 1978).

Horizontal gene transfer was detected across *B. canariense* and *B. japonicum* at the symbiotic *nifH* and *nodC* loci, which map more than 250 kb apart one from the other on the chromosomal symbiotic region of *B. japonicum* USDA 110 (Göttfert *et al.*, 2001; Kaneko *et al.*, 2002). The *nifH* and *nodC* phylogenies correlated well with the host range of the ECGL isolates (Jarabo-Lorenzo *et al.*, 2003; Vinuesa *et al.*, 2005), but were incongruent with the maximum-likelihood species phylogeny (Felsenstein, 2004; Nichols, 2001) inferred from combined and congruent *glnII* plus *recA* (compare Figs C and D, available as supplementary material in IJSEM Online) partitions (Vinuesa *et al.*, 2005). Regardless of their geographical origin and (geno)species assignation, all isolates from genistoid legumes and *Ornithopus* spp. contained *nifH* and *nodC* alleles that were recovered in highly supported clades in the corresponding gene trees (see Fig. Da and Db in IJSEM Online), highlighting the independent evolutionary histories of adaptative (accessory) and housekeeping (core) loci (Lan & Reeves, 2000; Wernegreen & Riley, 1999). The most parsimonious explanation for the observed phylogenetic incongruence between housekeeping and *sym* loci is that lateral transfer events of symbiotic islands took place across species, probably mediated by an illegitimate recombination mechanism (Kaneko *et al.*, 2002; Sullivan & Ronson, 1998). Therefore, phylogenetic analysis of these two symbiotic genes, coupled with host-range experiments (Table 1), allowed us to uncover and delineate

Table 1. Host-range experiments performed in Leonard jars using selected *Bradyrhizobium* strains and legume hosts

Bradyrhizobium* species, biovar and strain	**Host†**					
	C. proliferus	***T. stenopetala***	***L. luteus***	***G. max***	***G. soja***	***M. atropurpureum***
B. canariense bv. *genistearum* BTA-1^T	Fix$^+$‡	Fix$^+$	Fix$^+$	Nod$^-$	Nod$^-$	Fix$^{+/-}$
B. canariense bv. *genistearum* BC-C2	Fix$^+$	Fix$^+$	Fix$^+$	Nod$^-$	Nod$^-$	Fix$^{+/-}$
B. canariense bv. *genistearum* BC-MAM1	Fix$^+$	Fix$^+$	Fix$^+$	Nod$^-$	Nod$^-$	Fix$^{+/-}$
B. canariense bv. *genistearum* ISLU16	Fix$^+$	Fix$^+$	Fix$^+$	Nod$^-$	Nod$^-$	ND
B. japonicum bv. *genistearum* BGA-1	Fix$^+$	Fix$^+$	Fix$^+$	Nod$^-$	Nod$^-$	Fix$^{+/-}$
B. japonicum bv. *genistearum* FN13	Fix$^+$	Fix$^+$	Fix$^+$	Nod$^-$	Nod$^-$	ND
B. japonicum bv. *genistearum* Blup-MR1	Fix$^+$	Fix$^+$	Fix$^+$	Nod$^-$	Nod$^-$	ND
B. japonicum bv. *glycinearum* USDA 110	Nod$^-$	Nod$^-$	Nod$^-$	Fix$^+$	Fix$^+$	Fix$^+$
B. japonicum bv. *glycinearum* DSM 30131^T	Nod$^-$	Nod$^-$	Nod$^-$	Fix$^+$	Fix$^+$	Fix$^{+/-}$
B. liaoningense bv. *glycinearum* LMG 18230^T	Nod$^-$	Nod$^-$	Nod$^-$	Fix$^+$	ND	ND
B. yuanmingense CCBAU 10071^T	Nod$^-$	Nod$^-$	Nod$^-$	Nod$^-$	ND	ND

*Species and biovar assignation of strains is supported by the ITS, *glnII*+*recA*, *niH* and *nodC* phylogenies presented in Figs B, C and D (available as supplementary material in IJSEM Online) and further evidence presented in Vinuesa *et al.* (2005).

†*C. proliferus*, *T. canariense*, *L. luteus*, *G. max*, *G. soja* and *M. atropurpureum* are species of the genera *Chamaecytisus*, *Teline*, *Lupinus*, *Glycine* and *Macroptilium*, respectively.

‡Fix$^+$ indicates a nitrogen-fixing symbiosis, as revealed by the acetylene reduction assay; Fix$^{+/-}$ indicates weak levels of acetylene reduction; Nod$^-$ indicates a non-nodulating interaction; ND, not determined. Plant germination, inoculation and cultivation were as described previously (Vinuesa *et al.*, 1998).

for the first time *Bradyrhizobium* biovarieties (symbiotic ecotypes, Fig. D in IJSEM Online), as defined in Vinuesa *et al.* (2005), and according to the proposed minimal standards for the description of new genera and species of root- and stem-nodulating bacteria (Graham *et al.*, 1991). These biovarieties should not be confounded with new species on the basis of their symbiotic (host range) phenotypes (Graham *et al.*, 1991; Lan & Reeves, 2001). We agree with Graham *et al.* (1991) that species descriptions of symbiotic rhizobia should be accompanied by a definition of their biovariety in the form of a latin trinomial, although we disagree with the proposal of equating each ecotype with a new species (Cohan, 2002), since the ecological characters conferred by symbiotic plasmids or islands are highly prone to rapid gain and loss events and horizontal transfer, and well delineated evolutionary species, such as *B. japonicum*, have more than one biovariety (see Fig. Da and Db in IJSEM Online).

An estimate of the *Bradyrhizobium* species phylogeny (Felsenstein, 2004; Nichols, 2001) based on a maximum-likelihood analysis of congruent *glnII*+*recA* sequence partitions (Fig. C in IJSEM Online) provided strong evidence that *B. canariense* is the sister species of *B. japonicum*, which is consistent with a Bayesian phylogeny presented elsewhere (Vinuesa *et al.*, 2005). This species phylogeny is congruent with the current taxonomy of the genus. Only the position of *B. betae* remains uncertain because *rrs* and ITS sequences do not resolve its phylogenetic placement (see Figs B and E, available as supplementary material in IJSEM Online) and protein-coding gene sequences are not available for this taxon yet. Importantly, the *glnII*+*recA* species phylogeny (see Fig. C and the final and concluding remarks in IJSEM Online) does not provide conclusive support to the hypotheses derived from numerical taxonomy and *rrs* sequence analyses that the photosynthetic bradyrhizobia and *B. elkanii* may represent new genera (Ladha & So, 1994; van Berkum & Eardly, 1998; Willems *et al.*, 2001b). Taking a more conservative classification criterion based on a consensus of the different data sources available at the moment for these bacteria, and considering the topology presented in Fig. C, favours their classification as basal lineages of the genus *Bradyrhizobium* (see the final and concluding remarks in IJSEM Online), which supports the conclusions reached by So *et al.* (1994) based on *rrs* and fatty acid analysis that they are bradyrhizobia, as well as the old hypothesis of a photosynthetic ancestor for the genus (Jarvis *et al.*, 1986; Vinuesa *et al.*, 2005).

It had been shown previously that ITS sequence clades correlate reasonably well with DNA-homology groups (Willems *et al.*, 2001a, 2003). Therefore, we used the topologies inferred from the ITS and *glnII*+*recA* sequence data (Figs B and C in IJSEM Online) to select a number of representative *B canariense*, *B. japonicum* and *B. liaoningense* strains to perform DNA–DNA hybridization experiments, using a filter hybridization (dot-blot) technique. Three replicate samples of 2 μg of purified genomic DNA (genomic DNA purification kit; Roche Molecular Biochemicals) were vacuum-blotted onto nylon membranes and cross-linked with UV light. Five-hundred nanograms of purified genomic DNA from three distinct *B. canariense* strains (BC-C2, BES-1 and BTA-1^T) were randomly labelled with digoxigenin using the DIG-labelling system (Roche

Table 2. Percentage of relative DNA–DNA hybridization obtained between *Bradyrhizobium canariense* BC-C2, BES-1 and BTA-1^T, conspecific isolates and selected *B. japonicum* and *B. liaoningense* strains

Bradyrhizobium species, biovar and strain	DNA homology (%) with probe:			Avg.*
	BC-C2	BES-1	BTA-1^T	
B. canariense bv. *genistearum* BC-C2	100	85±5	81±3	82·9±9·5, *n*=18
B. canariense bv. *genistearum* BES-1	74±4	100	88±4	
B. canariense bv. *genistearum* BTA-1^T	70±3	85±3	100	
B. canariense bv. *genistearum* BC-P1	76±3	84±4	84±4	
B. canariense bv. *genistearum* BC-P5	69±1	81±3	80±2	
B. canariense bv. *genistearum* BC-MAM1	84±3	73±4	78±5	
B. japonicum bv. *genistearum* BGA-1	38±6	41±2	46±4	34·9±10·6, *n*=12
B. japonicum bv. *glycinearum* DSM 30131^T	24±3	41±4	39±2	
B. japonicum bv. *glycinearum* USDA 110	13±6	34±4	30±7	
B. japonicum bv. *glycinearum* X6-9	22±4	47±3	44±4	
B. liaoningense bv. *glycinearum* LMG 18230^T	25±5	48±9	37±6	33·2±10·4, *n*=6
B. liaoningense Spr3-7	19±3	39±8	31±6	

*Avg., average percentage of DNA hybridization signal among strains for the number (*n*) of comparisons indicated.

Molecular Biochemicals) and used as probes (adjusted to 20 ng ml^{-1} in the hybridization solution). Stringent hybridization was carried out overnight at 68 °C, followed by high-stringency washings at 68 °C in 0·5× SSC. Hybridization signals were detected by chemiluminiscence using anti DIG Fab fragments and the enhanced chemifluorescence substrate (Roche Molecular Biochemicals), and quantified using a Storm 860 phosphorimager (Molecular Dynamics) equipped with the ImageQuant software (Amersham Pharmacia Biotech). The hybridization results are shown in Table 2 and indicated that the three probes hybridized significantly stronger (in the 69–88 % range) with *B. canariense* isolates than with *B. japonicum* and *B. liaoningense* strains (13–48 % range).

B. canariense strains can be further distinguished from all other described *Bradyrhizobium* species by a combination of genotypic, physiological and ecological traits, as indicated in the species description. Distinctive phenotypic features of *B. canariense* are presented in Table 3. It should be noted, however, that such phenotypic traits are highly inconsistent when large populations are studied (Xu *et al.*,

Table 3. Distinctive phenotypic features for *Bradyrhizobium canariense* and reference *Bradyrhizobium* species

Species: 1, *B. canariense*; 2, *B. japonicum*; 3, *B. elkanii*; 4, *B. liaoningense*; 5, *B. yuanmingense*; 6, *B. betae*. Data for *B. japonicum*, *B. elkanii* and *B. yuanmingense* were taken from Table 3 of Yao *et al.* (2002), data for *B. liaoningense* from Xu *et al.* (1995), and data for *B. betae* from Rivas *et al.* (2004). +, >95 % of isolates were positive; −, >95 % of the isolates were negative; +/−, variable; NR, not reported.

Characteristics	1	2	3	4	5	6
C sources:						
D-Fructose	+	−*	+	+/−	−	+/−
Lactose	−	+*	+/−	−	−	−
Maltose/sucrose	−	+*	+*	−	+/−	+/−
N source:						
L-Glycine	−	+*	+*	NR	−	NR
Resistance to:						
Erythromycin (100 μg ml^{-1})	+/−	−*	+	NR	−	+/−
Growth characteristics:						
pH 4·5	+	−	+	NR	−	NR
pH 10	−	−	+/−	−	−	NR
1·0 % NaCl	−	+*	+*	−	−	+
Colony size (mm) after 7 days incubation in YMA	1·0–1·5	1·0	1·0	0·2–1·0	1·0–2·0	NR
Generation time (h) in YM broth, pH 6·8	>6	>6	>6	16–40	9·5–16	12–16

*Our own data are not consistent with those reported previously.

1995; Yao *et al.*, 2002), and it is well documented that phenotypic and genotypic clustering of *Bradyrhizobium* strains correlates poorly (Dupuy *et al.*, 1994; So *et al.*, 1994; van Rossum *et al.*, 1995; Zhang *et al.*, 1999).

The description of *Bradyrhizobium canariense* sp. nov. is therefore supported by the population genetics, phylogenetic, DNA homology, physiological and ecological evidence presented above. This is the first description of a novel root nodule microsymbiont species that is primarily based on molecular evolutionary criteria, using a large collection of strains from different hosts and geographical origins, which have been extensively characterized by a broad range of genotyping methods (REP-PCR, *rrs*+ITS PCR-RFLPs, MLEE and stable low-molecular-weight RNAs), as well as by state-of-the-art phylogenetic methods using seven gene partitions [five informational/housekeeping loci (*rrs*, ITS, *atpD*, *glnII* and *recA*) and two *sym* loci (*nifH* and *nodC*)]. Therefore, this work follows the recommendations made by the ad hoc committee for the re-evaluation of the species definition in bacteriology (Stackebrandt *et al.*, 2002). Furthermore, it augments and actualizes the proposed minimal standards for the description of new genera and species of root- and stem-nodulating bacteria (Graham *et al.*, 1991) by the use of more advanced analytical tools, a highly resolved *Bradyrhizobium* species phylogeny and an updated theoretical framework. Finally, in view of the richness of evolutionary and ecological inferences that can be made from sequence data (see the final and concluding remarks provided in IJSEM Online), we would like to encourage (brady)rhizobial taxonomists to make more extensive use of them in future works. In doing so, a large multilocus sequence database could be built up quickly and used as the primary source of characters for molecular evolutionary and systematic studies. Only sequence data are highly portable and freely exchangeable by different researchers for unambiguous comparative analyses.

Description of *Bradyrhizobium canariense* sp. nov.

Bradyrhizobium canariense [ca.na.ri.en′se. N.L. neut. adj. *canariense* pertaining to the Canary Islands (Islas Canarias), where it is the dominant species nodulating endemic shrub legumes Papilionoideae: Genisteae].

Gram-negative, aerobic, slow-growing, non-spore-forming rods, as for other species of the genus, motile by a single subpolar flagellum (León-Barrios *et al.*, 1991). Phenotypically, *B. canariense* strains are highly diverse. Colonies on YMA (pH 6·8) are white or creamy, 1–1·5 mm in diameter after 7 days incubation at 28 °C, producing an acid reaction and variable amounts of exopolysaccharides, as reflected by the diverse textures, consistencies and growth patterns they exhibit on solid media. Their lipopolysaccharide (LPS) O-antigens are also highly diverse as determined by DOC-PAGE analysis of purified LPSs and immunological cross-reactions (León-Barrios *et al.*, 1991; Santamaría *et al.*, 1997). Optimum growth temperature is 28–30 °C, but inhibited at 37 °C. No growth is observed at pH 9, or in the presence of 1 % NaCl on YMA. They use (+)-D-glucose, (+)-D-mannose, (+)-D-galactose, (−)-D-fructose, (−)-L-rhamnose, (+)-D-xylose, (−)-D-ribose, (−)-D-arabinose, glycerol, mannitol, sorbitol, citrate, fumarate and succinate, but not (−)-L-sorbose, melibiose, lactose, sucrose, (+)-D-trehalose, inulin, starch or catechol as sole carbon sources. Use L-glutamine but not L-glycine as sole N source. They are highly acid-tolerant, forming colonies of 1 mm in diameter after 6 to 7 days incubation at 30 °C on acidified 20E plates at pH 4·2 solidified with GelRite (Roth, Germany) and buffered with 25 mM Homopipes (Vinuesa *et al.*, 2003). The symbiotic genes map to the chromosome, lacking plasmids as revealed by Eckhardt gel-electrophoresis (Eckhardt, 1978). Its known geographical distribution includes Spain, Morocco, the Canary Islands and the Americas. A single biovariety (bv. *genistearum*) is presently known, which nodulates different genera and species in the legume tribe Genisteae (e.g. *Lupinus* spp., *Adenocarpus* spp., *Chamaecytisus proliferus*, *Spartocytisus supranubius* and *Teline* spp.), as well as *Ornithopus* spp. (Papilionoideae: Loteae), but does not nodulate soybeans (*Glycine max* or *Glycine soja*, Papilionoideae: Phaseoleae). At the molecular level this species can be easily distinguished from strains of its sister species *B. japonicum* and all other described *Bradyrhizobium* species by the unique 16S rRNA PCR-RFLP genotype obtained with the endonucleases *Hha*I, *Dde*I and *Hin*fI (Jarabo-Lorenzo *et al.*, 2000, 2003; Vinuesa *et al.*, 1998, 1999, 2005). *B. canariense* strains also display a distinct fingerprint of stable low-molecular-weight RNAs (Jarabo-Lorenzo *et al.*, 2000). It forms statistically highly supported ITS, *atpD*, *glnII* and *recA* sequence clades under the maximum-likelihood optimality criterion using best-fit models of nucleotide substitution (with bootstrap support >90 % in all cases). *B. canariense* strains are only weakly clonal, with significant recombination taking place within populations. DNA homology is greater than 69 % between *B. canariense* strains, and lower than 50 % with *B. japonicum* or *B. liaoningense* strains, its closest phylogenetic relatives.

The type strain, BTA-1^T (=ATCC BAA-1002^T=LMG 22265^T=CFNE 1008^T), was isolated from the root nodules of *Chamaecytisus proliferus* subsp. *proliferus* var. palmensis (Papilionoideae: Genisteae) in La Laguna, Tenerife, Canary Islands, Spain, and has a G+C content of 63·8 mol%. This and other *B. canariense* strains have been deposited at the strain collections of the CIFN-UNAM and the Departments of Microbiology at the Universities of La Laguna and Gent, from where they are freely available.

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