Strains of Bradyrhizobium cosmicum sp. nov., isolated from contrasting habitats in Japan and Canada possess photosynthesis gene clusters with the hallmark of genomic islands

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Abstract

The taxonomic status of two previously characterized Bradyrhizobium strains (58S1T and S23321) isolated from contrasting habitats in Canada and Japan was verified by genomic and phenotypic analyses. Phylogenetic analyses of five and 27 concatenated protein-encoding core gene sequences placed both strains in a highly supported lineage distinct from named species in the genus Bradyrhizobium with Bradyrhizobium betae as the closest relative. Average nucleotide identity values of genome sequences between the test and reference strains were between 84.5 and 94.2 %, which is below the threshold value for bacterial species circumscription. The complete genomes of strains 58S1T and S23321 consist of single chromosomes of 7.30 and 7.23 Mbp, respectively, and do not have symbiosis islands. The genomes of both strains have a G+C content of 64.3 mol%. Present in the genome of these strains is a photosynthesis gene cluster (PGC) containing key photosynthesis genes. A tRNA gene and its partial tandem duplication were found at the boundaries of the PGC region in both strains, which is likely the hallmark of genomic island insertion. Key nitrogen-fixation genes were detected in the genomes of both strains, but nodulation and type III secretion system genes were not found. Sequence analysis of the nitrogen fixation gene, nifH, placed 58S1T and S23321 in a novel lineage distinct from described Bradyrhizobium species. Data for phenotypic tests, including growth characteristics and carbon source utilization, supported the sequence-based analyses. Based on the data presented here, a novel species with the name Bradyrhizobium cosmicum sp. nov. is proposed with 58S1T (=LMG 31545T=HAMBI 3725T) as the type strain.

The genus Bradyrhizobium is a large and diverse group of bacterial species and includes members that possess accessory genes for nitrogen fixation, photosynthesis and/or symbiotic interaction with legume plants [1].

In a previous study [2], bacteria were isolated from root nodules of soybean plants that had been inoculated with root-zone soils from legumes native to Canada. Bacterial isolates were characterized by multiple locus sequence analysis (MLSA) of five protein-encoding core genes and several novel lineages in the genus Bradyrhizobium were identified. One of these novel lineages, represented by strain 58S1T, is a close relative of Bradyrhizobium betae PL7HG1T [3] that was recently reported to harbour key photosystem genes [4]. During the course of the present work we showed that strain 58S1T also possesses photosystem genes and, based on results of taxonomic analyses, is highly similar to Bradyrhizobium sp. S23321 [5], which was isolated from paddy field soil in Japan and has been subjected to detailed genomic analysis.
Here we used complete genome, phylogenetic and phenotypic analyses to further characterize strains 58S1T and S23321 and based on the results a novel species for which the name *Bradyrhizobium cosmicum* sp. nov. is proposed.

**HABITAT AND ISOLATION**

Novel strain 58S1T was isolated from a root nodule of a soybean plant that had been inoculated with a suspension of root-zone soil of *Amphicarpaea bracteata* (hog peanut) plants growing in deciduous woodland in Gatineau, Quebec, Canada [2]. Strain 58S1T was deposited in the BCCM/LMG Bacteria Collection, University of Ghent, Belgium (LMG collection no. 31545) and in the HAMBI Microbial Culture Collection, University of Helsinki, Finland (HAMBI collection no. 3725). Novel strain S23321 was isolated from paddy field soil at the experimental farm of Tohoku University, Japan [5] and was deposited in the Japan Collection of Microorganisms (JCM collection no. 18004).

**PHYLOGENETIC CHARACTERIZATION OF PARTIAL GENE SEQUENCES**

For phylogenetic analyses, sequences of 16S rRNA, *atpD*, *glnII*, *gyrB*, *recA* and *rpoB* core genes were used. Nucleotide sequence accession numbers are given in Table S1 (available in the online version of this article). Sequence alignments of protein-encoding core genes (*atpD*, *glnII*, *gyrB*, *recA* and *rpoB*) were carried out as previously described [6]. Alignment of 16S rRNA gene sequences was done using the fast, secondary-structure aware Infernal aligner version 1.1 using the online Ribosomal Database Project version 11.5 [7]. Best-fit substitution models were selected using ModelTest-NG [8] implemented in the cipres Science Gateway version 3.3 [9]. Maximum-likelihood (ML) phylogenetic analyses [10] were performed using 1000 non-parametric bootstrap replications to assess support [6]. Bayesian phylogenetic analyses were carried out using MrBayes version 3.2.1 with default priors [11] as detailed previously [12]. In all instances, tree topologies from Bayesian and ML analyses were similar and therefore only the Bayesian trees are shown.

In order to include all described species of the genus *Bradyrhizobium* in a phylogenetic analysis of 16S rRNA gene sequences, it was necessary to trim aligned sequence lengths to 1300 bp. The 16S rRNA gene tree (Fig. S1) shows that novel strains 58S1T and S23321 possess identical 16S rRNA gene sequences and were placed in a superclade represented by *Bradyrhizobium japonicum* with the type strain of *B. betae* their as closest relative. Sequence similarities of the 16S rRNA gene of these *Bradyrhizobium* species versus 58S1T and S23321 (Table S2), calculated using software implemented in EzBioCloud [13], are consistent with the phylogenetic data indicating that *B. betae* is the closest relative. It should be noted, however, that the 16S rRNA gene is highly conserved and its usefulness as a taxonomic marker for species delineation in the genus *Bradyrhizobium* is limited [14, 15].

**GENOMIC CHARACTERIZATION**

The complete genome of strain 58S1T was sequenced at the Genome Quebec Innovation Centre, Montreal, Canada, using the Pacific Biosciences (PacBio) RS II single-molecule real-time (SMRT) platform [20] as described previously [21]. Estimated genome coverage for strain 58S1T was 136-fold with 104918 polymerase reads and an average read length of 13 299 bp. The complete genome sequence of S23321 was determined in a previous study [5] by a whole genome shotgun approach using hybrid assembly of Sanger end sequences consisting of 3 kb and 10 kb clone libraries (53760 reads, 4.5-fold genome coverage) and 454 pyrosequencing data with appropriate gap filling procedures.

The complete genomes of strains 58S1T and S23321 consist of single circular chromosomes of size 7304136 bp and...
Fig. 1. Bayesian phylogenetic tree (GTR+G+I substitution model) of atpD–glnII–recA–gyrB–rpoB concatenated housekeeping gene sequences for *Bradyrhizobium cosmicum* sp. nov. and reference taxa of the genus *Bradyrhizobium*. Alignment lengths: atpD, 429 bp; glnII, 519 bp; recA, 417 bp; gyrB, 600 bp; rpoB, 714 bp; total, 2679 bp. Posterior probabilities ≥0.90 are shown. Bar, expected substitutions per site.
Fig. 2. Bayesian phylogenetic tree (HKY+G substitution model) of nifH gene sequences (540 bp) for Bradyrhizobium cosmicum sp. nov. and reference taxa of the genus Bradyrhizobium. Posterior probabilities ≥0.90 are shown. Bar, expected substitutions per site.
These genomes are similar in size to close relative, *B. betae* PL7HG1T, with a complete genome of size 7,419,402 bp (Table 1). However, unlike *B. betae* PL7HG1T, novel strains 58S1T and S23321 do not possess plasmids [4].

The DNA G+C content of strains 58S1T and S23321 is 64.3 mol%, which is within the range for members of the genus *Bradyrhizobium*. Totals of 6930 coding sequences, 48 tRNAs and a single rRNA operon were found for strain 58S1T using the NCBI Prokaryotic Genome Annotation Pipeline version 4 [22, 23]. For strain S23321, 6983 coding sequences, 45 tRNAs and a single rRNA operon were detected using the PATRIC version 3.5.26 platform [24].

The most abundant genes for strains 58S1T and S23321, respectively, are those involved in metabolism (1045 and 1007 genes), energy (310 and 301 genes), protein processing (235 and 229 genes), membrane transport (231 and 246 genes) and cellular processes (175 and 160 genes). Genes involved in motility and chemotaxis, stress response (heat/cold and osmotic shock), resistance to antibiotics and toxic compounds were also detected in both 58S1T and S23321.

Average nucleotide identity (ANI) as an overall genome relatedness index is recommended to replace DNA–DNA hybridization methods for bacterial species delineation [14, 15, 25, 26]. We estimated ANI values for the complete genome sequence of 58S1T and S23321 in pairwise comparisons with genome sequences of type strains of described *Bradyrhizobium* species available in public databases using the MUMmer (ANIm) algorithm implemented in the Jpecies web server version 3.0.20 [27]. Table 2 shows that, compared to 58S1T and S23321, ANI values varied between 84.4% (*B. retamae* Ro19T) and 94.2% (*B. betae* PL7HG1T), which is below the accepted threshold value of 95–96% for bacterial species circumscription [14, 25, 28]. In contrast, the ANI value of 97.9% for the comparison of novel strains 58S1T versus S23321 is consistent with these strains belonging to the same species. These data are also in accord with the phylogenetic results (Fig. 1) indicating that *B. betae* PL7HG1T is the closest relative of novel strains 58S1T and S23321.

Phylogenomic relationships were investigated employing amino acid sequences of 27 conserved marker genes obtained from the genomes of 41 type strains of *Bradyrhizobium* species available in public databases using AmphoraNet [29], a web-based implementation of amphora2 [30]. Sequences were aligned with Muscle [31] and then processed with TrimAl [32] to remove poorly aligned regions. Alignments were concatenated and the best-fit amino acid substitution model was selected using ModelTest-NG [8]. The placement of taxa in a Bayesian phylogenetic tree (Fig. 3) corroborates our finding that the closest relative of novel strains 58S1T and S23321 is *B. betae* PL7HG1T. Fig. 3 also shows that the 41 *Bradyrhizobium* type strains were divided into four highly supported ‘superclades’ represented by type strains of

Table 1. Characteristics of genome sequences of *Bradyrhizobium cosmicum* sp. nov., strains 58S1T (accession no. CP041656) and S23321 (accession no. AP012279) and close relatives, *Bradyrhizobium betae* PL7HG1T (accession no. CP044543), *Bradyrhizobium diazoefficiens* USDA 110T (accession no. CP011360) and *Bradyrhizobium japonicum* USDA 6T (accession no. AP012206).

| Characteristic                        | Strain          |
|--------------------------------------|-----------------|
|                                      | 58S1T           | S23321          | PL7HG1T          | USDA110T          | USDA6T           |
| Genome assembly quality              | Complete        | Complete        | Complete         | Complete          | Complete         |
| Genome size (bp)                     | 7,304,136       | 7,231,841       | 7,419,402        | 9,105,828         | 9,207,384        |
| CDS (total)                          | 6,930           | 6,983†          | 7,113            | 8,489             | 9,447*†          |
| CDS (coding)                         | 6,757           | 6,898†          | 6,780            | 8,220             | 8,829†‡          |
| rRNAs                                | 3               | 3               | 3                | 3                 | 6†               |
| tRNAs                                | 48              | 45†             | 47               | 52                | 51‡              |
| Pseudo genes (total)                 | 173             | 122†            | 333              | 213               | NA               |
| Repeat regions                       | 58*             | NA              | 58*              | 148*              | 128*             |
| DNA G+C content (mol%)               | 64.3*           | 64.3†           | 64.8§            | 64.1*             | 63.7†            |
| Photosynthesis gene cluster          | Yes             | Yes             | Yes              | No                | No               |
| Plasmids                             | 0               | 0               | 1                | 0                 | 0                |
| Symbiosis island                     | No              | No              | No               | Yes               | Yes              |

*Data from the PATRIC Bioinformatics Database [24].
†Data from Okubo et al. 2012 [5].
‡Data from Kaneko et al. 2011 [49].
§Data from Cloutier and Bromfield 2019 [4].
Table 2. Average nucleotide identity (ANI) values for pairwise comparisons of genome sequences of *Bradyrhizobium cosmicum* sp. nov 58S1\textsuperscript{T} and S23321 versus *Bradyrhizobium* species in public databases

| Reference strain (accession no.) | ANI (%) | Reference strain (accession no.) | ANI (%) |
|----------------------------------|---------|----------------------------------|---------|
|                                   | 58S1\textsuperscript{T} | S23321                           | 58S1\textsuperscript{T} | S23321 |
| *Bradyrhizobium cosmicum* 58S1\textsuperscript{T} (CP041656) | –       | 97.9                             | 87.7 | 87.8 |
| *Bradyrhizobium cosmicum* S23321 (AP012279) | 97.9     | –                                | 87.7 | 87.7 |
| *Bradyrhizobium betae* PL7HG1\textsuperscript{T} (CP044543) | 94.1     | 94.2                             | 87.7 | 87.7 |
| *Bradyrhizobium diazoefficiens* USDA 110\textsuperscript{T} (CP011360) | 89.4     | 89.4                             | 87.6 | 87.6 |
| *Bradyrhizobium japonicum* USA 6\textsuperscript{T} (AP012206) | 89.4     | 89.4                             | 85.3 | 85.3 |
| *Bradyrhizobium nifali* CNPS0 3448\textsuperscript{T} (SPQ00000000) | 89.4     | 89.4                             | 85.3 | 85.3 |
| *Bradyrhizobium styloanthis* BR 446\textsuperscript{T} (LVE000000000) | 88.8     | 88.9                             | 85.3 | 85.3 |
| *Bradyrhizobium arachidis* CCBAU 051107\textsuperscript{T} (FPBP000000000) | 88.8     | 88.8                             | 85.3 | 85.3 |
| *Bradyrhizobium symbiodificiens* 85S1MB\textsuperscript{T} (CP029427) | 88.8     | 88.8                             | 85.2 | 85.2 |
| *Bradyrhizobium ottawaense* OO99\textsuperscript{T} (CP029425) | 88.8     | 88.8                             | 85.2 | 85.2 |
| *Bradyrhizobium shewense* ERR11\textsuperscript{T} (FMA000000000) | 88.7     | 88.8                             | 85.2 | 85.2 |
| *Bradyrhizobium amphicarpae* 39S1MB\textsuperscript{T} (CP029426) | 88.6     | 88.6                             | 85.2 | 85.2 |
| *Bradyrhizobium nitroreducens* TSA1\textsuperscript{T} (LFJ000000000) | 88.6     | 88.6                             | 84.9 | 84.9 |
| *Bradyrhizobium frederickii* CNPS0 3426\textsuperscript{T} (SPQ000000000) | 88.6     | 88.6                             | 84.7 | 84.7 |
| *Bradyrhizobium zhanjiangense* CCBAU 51778\textsuperscript{T} (CP022221) | 88.6     | 88.6                             | 84.7 | 84.7 |
| *Bradyrhizobium forestalis* INPA54B\textsuperscript{T} (PGVG000000000) | 88.6     | 88.6                             | 84.7 | 84.7 |
| *Bradyrhizobium sacchari* BR 10280\textsuperscript{T} (LWIG000000000) | 88.5     | 88.5                             | 84.7 | 84.7 |
| *Bradyrhizobium nanningense* CCBAU 53390\textsuperscript{T} (LBJC000000000) | 88.4     | 88.4                             | 84.7 | 84.7 |
| *Bradyrhizobium vignae* 7-2\textsuperscript{T} (RDQF000000000) | 88.3     | 88.3                             | 84.6 | 84.6 |
| *Bradyrhizobium guangxiense* CCBAU 53363\textsuperscript{T} (CP022219) | 88.3     | 88.3                             | 84.4 | 84.4 |
| *Bradyrhizobium yangningense* CCBAU 10071\textsuperscript{T} (FMAE000000000) | 88.2     | 88.2                             | 84.5 | 84.5 |
| *Bradyrhizobium guangzhouense* CCBAU 51670\textsuperscript{T} (CP030053) | 87.8     | 87.8                             | 84.5 | 84.5 |
Fig. 3. Bayesian phylogenetic tree (JTT +G+I substitution model) inferred from 27 concatenated protein encoding gene sequences for strains of *Bradyrhizobium cosmicum* sp. nov. and type strains of 41 *Bradyrhizobium* species. Alignment lengths (amino acids): frr, 191; infC, 196; nusA, 536; pgk, 394; pyrG, 545; rplA, 232; rplB, 196; rplC, 244; rplE, 201; rplF, 178; rplK, 142; rplL, 124; rplM, 170; rplN, 128; rplP, 141; rpsS, 139; rpmA, 94; rpoB, 1030; rpsB, 342; rpsC, 237; rpsE, 193; rpsL, 158; rpsK, 130; rpsM, 131; rpsS, 96; smpB, 158; tsf, 308; total, 6634. Posterior probabilities ≥0.90 are shown. Bar, expected substitutions per site.
B. japonicum, B. oligotrophicum, B. elkanii and B. jicamae, with novel strains 58S1T and S23321 placed in the superclade represented by B. japonicum. These four superclades have also been identified in other phylogenetic studies of the genus Bradyrhizobium (e.g. [1, 33, 34]). It is noteworthy that the overall topology of the tree in Fig. 3 is consistent with the tree in Fig. 1, thereby validating the use of five concatenated core gene sequences for species delineation.

Further genomic analysis of novel strains was done using GenomeMatcher software [35]. Circular representation of genomes (Fig. 4a–c) shows that strain 58S1T has high similarity to S23321 throughout its genome whereas similarity of both novel strains to B. betae PL7HG1T is relatively low. These observations are consistent with expectations for comparisons within and between Bradyrhizobium species. Comparison with the genome of soybean-nodulating bacterium B. diazoefficiens USDA 110T [36], show that novel strains 58S1T and S23321, like close relative B. betae PL7HG1T, do not contain a symbiosis island that carries nodulation genes (nodDYABCSUIJ) or type III secretion systems (T3SS) genes required for symbiotic interaction with leguminous plants (Fig. 4d, Table 1). However, key genes for nitrogen fixation, including nifDKEN, nifH, nifA and fixABCX, were detected in the genomes of 58S1T and S23321 but not in the genome...
of *B. betae* PL7HG1<sup>T</sup>. It is noteworthy that the organization of the *nif–fix* gene cluster is highly conserved in novel strains 58S1<sup>T</sup> and S23321 (Fig. S8).

Further analyses show that the genomes of strains 58S1<sup>T</sup> and S23321 [5] contain, respectively, a photosynthesis gene cluster (PGC) of about 51 kb (coordinates, 1481046–1532017 bp and 1998217–2049181 bp). The PGC in both novel strains contains key photosynthesis genes encoding the light-harvesting protein beta and alpha subunits (*pufBA*) and reaction centre L, M and H subunits (*pufLM* and *puhA*). Genes coding for bacteriochlorophyll (*bchIDOCXYZGPFNBHLM*) and *acsF*, carotenoid (*crtIBCDEF*), photosynthesis repressor proteins (*ppsR1* and *ppsR2*) and bacteriophytochrome (*bphP*) are also present (Fig. 5).

The arrangement of genes in the PGC of 58S1<sup>T</sup> and S23321 is similar to that in *B. betae* PL7HG1<sup>T</sup> [4] isolated from a tumour on the roots of sugar beet [3], *B. amphicarpaeae* 39S1MB<sup>T</sup> [37] isolated from a root nodule of soybean [2] and to *Rhodopseudomonas palustris* CGA009 [38]. In contrast, the arrangement of the genes in the PGC of strains 58S1<sup>T</sup> and S23321 differs from *B. oligotrophicum* S58<sup>T</sup> [39], a Nod factor-independent symbiont of the semi-aquatic legume, *Aeschynomene indica* (Fig. 5).

Harr plot analysis [40] was employed to further characterize the genomic structure of strains 58S1<sup>T</sup>, S23321 and *B. betae* PL7HG1<sup>T</sup> using GenomeMatcher software [35]. The results, based on comparisons with 58S1<sup>T</sup>, reveal colinearity of genomes (i.e. similar genes in two strains are in the same relative positions in their genomes) except for the PGC regions which are markedly nonlinear (Fig. 6a, c). Although the IslandViewer 4 [41] suite of programs did not predict the PGCs of strains 58S1<sup>T</sup> and S23321 as conventional genomic islands (GIs; Fig. 4a, b), we detected a tRNA gene and its partial tandem duplication at the boundaries of the PGC region in both of these strains (Figs 5 and 6b) which may be the hallmark of flexible GIs [42, 43]. Flexible GIs of this type are typically acquired by non-homologous recombination in preferential insertion sites, such as tRNAs, and may leave behind telltale partial direct repeats [44].

It is noteworthy that we also detected a tRNA gene but not its partial repeat in the border region of the PGC of *B. betae* PL7HG1<sup>T</sup> and *B. amphicarpaeae* 39S1MB<sup>T</sup> [37] (Fig. 5).
Collectively these observations suggest that in some species of the genus *Bradyrhizobium*, the PGC region may act as a mobile genetic element with potential for the horizontal transfer of photosynthesis genes. Comparison of the G+C contents of genomes with PGCs of strains 58S1^T^, S23321, *B. betae* PL7HG1^T^ and *B. amphicarpaeae* 39S1MB^T^ did not reveal significant differences, suggesting that any horizontal transfer of the PGC region may have occurred between closely related bacterial species or alternatively was a distant evolutionary event [45].

**PHENOTYPIC CHARACTERIZATION**

Novel strains 58S1^T^ and S23321 produce colonies that are circular, convex, beige, translucent and <1 mm diameter after 7 days growth on yeast extract–mannitol (YEM) agar medium [6] at 28 °C. Bacterial cells are Gram-stain-negative based on the KOH method of Buck [46]. They produce an alkaline reaction on YEM agar after 21 days growth at 28°C that is typical of the genus *Bradyrhizobium*. Strains 58S1^T^ and S23321 did not produce pink-pigmented colonies on modified HM agar medium [39] after 7–14 days at 28°C under fluorescent and incandescent or natural daylight (14 h light, 10 h dark) in contrast to photosynthetic reference strains, *B. oligotrophicum* S58^T^, *B. denitrificans* IFAM1005^T^ and *Bradyrhizobium* sp. BTAi1. Cell morphology was investigated using transmission electron microscopy as described previously [5, 12]. Cells of 58S1^T^ and S23321 [5] are rod-shaped with sub-polar and lateral flagella. Cells of the type strain (Fig. S9) have an average cell size of 0.86×1.64 µm, which is consistent with the characteristics of the genus *Bradyrhizobium* [47].

Analysis of fatty acids was done using the Sherlock Microbial Identification System (Mini) version 6.0 and the rtsba6 database as described previously [12]. Table S3 shows that novel strain 58S1^T^ exhibited a fatty acid profile characteristic of the genus *Bradyrhizobium* [48] with a predominance of fatty acids C<sub>16:0</sub> and C<sub>18:1ω6c/C<sub>18:1ω7c</sub></sub> (summed feature 8).

Multiple phenotypic tests including carbon source utilization and chemical sensitivity assays were carried out using Biolog GEN III MicroPlates according to the manufacturer’s instructions. The results (Table S4) show that strain 58S1^T^ can be differentiated from close relative, *B. betae* PL7HG1^T^ as well as from type strains of *B. cytisi, B. rifense, B. canariense, B. japonicum* and *B. diazoefficiens* on the basis of several of these phenotypic tests.

Plant tests were carried out using modified Leonard jars as described previously [2, 5, 6] with *B. diazoefficiens* USDA110^T^ and *B. oligotrophicum* S58^T^ as reference strains. Based on the results of these tests, strains 58S1^T^ and S23321 did not elicit nodules on roots of *Macroptilium atropurpureum* ‘Siratro’ or *Aeschynomene indica*. Further tests showed that 58S1^T^ did not elicit nodules on soybean ‘AC Orford’ or *Amphicarpaea bracteata*. The fact that strain 58S1^T^ was originally isolated from a root nodule of soybean [2] suggests that it may be an opportunist that occasionally occupies root nodules.

Based on the phylogenetic, complete genome sequence and phenotypic data presented here, we propose that strains 58S1^T^ and S23321 represent a novel species named *Bradyrhizobium cosmicum* sp. nov.

**DESCRIPTION OF BRADYRHIZOBIUM COSMICUM SP. NOV.**

*Bradyrhizobium cosmicum* (cos’mi.cum. L. neut. adj. cosmicum, of the world, cosmopolitan).

Cells are Gram-stain-negative, aerobic, non-spore-forming rods (approx. 0.86 µm wide and 1.64 µm long) with sub-polar and lateral flagella. Colonies on YEM agar medium are
circular, convex, beige, translucent and <1 mm in diameter after 7 days at 28°C. Growth occurs at pH 5–10 (optimum, pH 7.0). Produces an alkaline reaction on YEM agar. The type strain grows at 10°C, optimal at 28°C, but no growth occurs at 37°C. Does not produce pink-pigmented colonies on modified HM agar medium when exposed to light–dark cycles. The type strain does not grow in the presence of 1 % (w/v) NaCl. Utilizes d-mannose, d-galactose, d-fucose, l-fucose, acetic acid, formic acid and four other carbon sources. Does not utilize d-sorbitol, d-glucose-6-PO4, d-fructose-6-PO4, d-aspartic acid, l-glutamic acid, pectin, quinic acid, citric acid, l-malic acid, l-malic acid, Tween 40, β-hydroxy-d,l-butyric acid, acid lactone, d-gluconic acid, mucic acid, d-saccharic acid, and four other chemical compounds. Susceptible to nalidixic acid, troleandomycin, lincomycin, nalidixic acid and four other chemical compounds.

Predominant fatty acids are C16:0 and C18:1ω6c/C18:0ω7c (summed feature 8). Does not elicit root nodules on Glycine max, Macroptilium atropurpureum, Amphicarpaea bracteata or Aeschynomene indica.

The type strain, 58S1T (=LMG 31545T=HAMBI 3725T), was isolated from a root nodule of a soybean plant that was inoculated with root-zone soil of Amphicarpaea bracteata (Hog peanut) growing in Canada. The type strain contains key photosystem and nitrogen-fixation genes but not nodulation or type III secretion system genes. The DNA G+C content of the type strain is 64.3 mol% and the genome size is 7.30 Mbp. GenBank/EMBL/DDBJ accession numbers for the complete genome and the 16S rRNA, atpD, gltII, recA, gyrB, rpoB and nifH gene sequences of the type strain are CP041656, KP768789, KP768557, KP768615, KP615104, KP768731, KP768673 and CP041656, respectively.

Funding information
Funding by Agriculture and Agri-Food Canada (grant no. J-002272) and JSPS KAKENHI (grant no.18H02112) is gratefully acknowledged.

Acknowledgements
The authors are thankful to Keith Hubbard of the Microscopy Centre, AAFC, Ottawa, Canada for preparing electron microscope images and to Eric Giraud, Laboratoire des Symbioses Tropicales et Méditerranéennes, Montpellier, France for supplying seed of Aeschynomene indica used in plant tests.

Conflicts of interest
The authors declare that there are no conflicts of interest.

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