Rhizobium tumorigenes sp. nov., a novel plant tumorigenic bacterium isolated from cane gall tumors on thornless blackberry

Nemanja Kuzmanović, Kornelia Smalla, Sabine Gronow & Joanna Puławska

Four plant tumorigenic strains 932, 1019, 1078\(^T\) and 1081 isolated from cane gall tumors on thornless blackberry (Rubus sp.) were characterized. They shared low sequence identity with related Rhizobium spp. based on comparisons of 16S rRNA gene (≤98%) and housekeeping genes atpD, recA and rpoB (<90%). Phylogenetic analysis indicated that the strains studied represent a novel species within the genus Rhizobium, with Rhizobium tubonense CCBAU 85046\(^T\) as their closest relative. Furthermore, obtained average nucleotide identity (ANI) and in silico DNA–DNA hybridization (DDH) values calculated for whole-genome sequences of strain 1078\(^T\) and related Rhizobium spp. confirmed the authenticity of the novel species. The ANI-Blast (ANIb), ANI-MUMmer (ANIm) and in silico DDH values between strain 1078\(^T\) and most closely related R. tubonense CCBAU 85046\(^T\) were 76.17%, 84.11% and 21.3%, respectively. The novel species can be distinguished from R. tubonense based on phenotypic and chemotaxonomic properties. Here, we demonstrated that four strains studied represent a novel species of the genus Rhizobium, for which the name Rhizobium tumorigenes sp. nov. is proposed (type strain 1078\(^T\) = DSM 104880\(^T\) = CFBP 8567\(^T\)). R. tumorigenes is a new plant tumorigenic species carrying the tumor-inducing (Ti) plasmid.

Plant tumorigenic bacteria belonging to the family Rhizobiaceae are associated with crown gall and cane gall diseases that can affect various plants\(^1\)–\(^3\). The presence of a large conjugal tumor-inducing (Ti) plasmid in the genome of the host strain is essential for pathogenicity. So far, tumorigenic strains have been identified within multiple species of the genus Agrobacterium, as well as within species Allorhizobium vitis (i.e. Agrobacterium biovar 3/Agrobacterium vitis) and Rhizobium rhizogenes (i.e. Agrobacterium biovar 2/Agrobacterium rhizogenes).

Rubus spp. have been identified as natural hosts of tumorigenic Rhizobiaceae strains. Crown gall disease that was mostly associated with tumorigenic strains of R. rhizogenes and A. tumefaciens species complex (i.e. Agrobacterium biovar 1/Agrobacterium tumefaciens), including recently described species Agrobacterium arsenijevicii has been frequently reported on Rubus spp.\(^4\)–\(^12\). In general, crown gall disease symptoms include formation of tumors on roots and crowns of infected plants. In addition, tumorigenic R. rhizogenes strains were also isolated from aerial tumors formed at pruning wounds of blackberry-raspberry (Rubus occidentalis-Rubus idaeus) hybrid of cv. Lochness\(^6\). On the other hand, cane gall disease is characterized by formation of tumors on the cane surface that may increase in size and number and completely girdle affected cane sections in advanced stages of disease\(^1\). Although Agrobacterium rubi was initially recognized as a causal agent of cane gall disease of Rubus spp.\(^1\)–\(^6\), later reports on this disease are limited or entirely lacking.

In this study, we observed plants of thornless blackberry (Rubus sp.) showing cane gall symptoms corresponding to those described before by Hildebrand\(^1\), that originated from two plantations in western Serbia. Although disease developed repeatedly every year, it was not lethal for infected blackberry plants nor caused significant losses in yield. Here, we characterized atypical tumorigenic strains isolated from cane gall tumors by using a polyphasic taxonomic approach and demonstrated that they represent a novel tumorigenic species within the genus Rhizobium.

1 Julius Kühn-Institut, Federal Research Centre for Cultivated Plants (JKI), Institute for Epidemiology and Pathogen Diagnostics, Messewege 11-12, 38104, Braunschweig, Germany. 2 Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Inhoffenstrasse 7B, 38124, Braunschweig, Germany. 3 Research Institute of Horticulture, Konstytucji 3 Maja 1/3, 96-100, Skierniewice, Poland. Correspondence and requests for materials should be addressed to N.K. (email: kuzmanovic1306@gmail.com)
Figure 1. Maximum likelihood tree based on partial sequence of 16S rRNA gene (1273 bp) indicates the phylogenetic position of *Rhizobium tumorigenes* sp. nov. strains 932, 1019, 1078T and 1081 (marked in bold) and their relationship with related members of the *Rhizobiaceae* family. The tree was constructed using a general time reversible substitution model with a gamma distribution and invariant sites (GTR + G + I). Bootstrap values (expressed as a percentage of 1000 replications) equal to or higher than 60% are shown at nodes. *Bradyrhizobium japonicum* USDA 6T was used as the outgroup organism. DDBJ/EMBL/GenBank accession numbers are given in Table S3. The scale bar represents the estimated number of nucleotide substitutions per site.
Results and Discussion

Four atypical strains isolated from thornless blackberry showing cane gall symptoms, originating from two localities in western Serbia, were characterized by using polyphasic taxonomic methods. The strains studied possessed identical 16S rRNA gene sequences (calculated for the length of 1309bp). Furthermore, strains originating from the same locality (932/1019 and 1078T/1081) possessed identical sequences of genes. On the other hand, strains 932 and 1019 had high sequence identities (>97.5%) with strains 1078T and 1081 based on analysis of partial sequences of \textit{atpD} (496bp), \textit{recA} (541 bp) and \textit{rpoB} (585 bp) housekeeping genes (Table S1), suggesting that they are closely related and belong to the same species. The strains exhibited different PCR MP fingerprints (Fig. S1), which excluded the possibility of their clonal origin. However, strains originating from the same locality showed similar fingerprints, differing by several bands (Fig. S1).

The strains studied shared 16S rRNA gene sequence identity ≤98% with related \textit{Rhizobium} spp. (Table S1). It is notably low value, taking into account 16S rRNA gene sequence identities between related \textit{Rhizobium} species being above 99%, and in some cases even 100%, as it was shown, for example, for \textit{Rhizobium laguerreae} and \textit{Rhizobium leguminosarum} or \textit{Rhizobium aegyptiacum}, \textit{Rhizobium bangladeshense} and \textit{Rhizobium binae}.

Moreover, nucleotide identity values were remarkably low (<90%) when comparing \textit{atpD}, \textit{recA} and \textit{rpoB} gene sequences of novel strains and related species (Table S1).

Based on 16S rRNA gene phylogeny, strains studied were grouped within the genus \textit{Rhizobium}, however, they formed a separate cluster, with \textit{Rhizobium tumonense} as their closest relative (Fig. 1). For further phylogenetic analysis, we selected species closely related to novel strains and included representative members of the \textit{Rhizobiaceae} family. Phylogenetic trees generated by using partial sequences of \textit{atpD}, \textit{recA} and \textit{rpoB} genes confirmed independent clustering of the novel strains with \textit{R. tumonense} CCBAU 85046\textsuperscript{T} located on a neighbouring branch (Fig. 2).

The draft genome sequence of \textit{R. tumonense} 1078\textsuperscript{T} consisted of 5,899,412 bp (129 contigs) with an average coverage of 127.6x. For \textit{R. tumonense} CCBAU 85046\textsuperscript{T}, the assembly generated 85 contigs comprising of 6,540,512 bp with an average coverage of 131.8x. \textit{R. tumonense} 1078\textsuperscript{T} and \textit{R. tumonense} CCBAU 85046\textsuperscript{T} had similar average GC contents of 60.0% and 59.3%, respectively, which was generally in accordance with other related \textit{Rhizobium} spp., e.g. \textit{R. rhizogenes} ATCC 11325\textsuperscript{T} (59.9%), \textit{Rhizobium tropici} CIAT 899\textsuperscript{T} (59.9%) or \textit{Rhizobium freirei} PRF 81\textsuperscript{T} (59.9%).

Genome-wide phylogeny based on 385 conserved proteins further supported distinctiveness of representative strain 1078\textsuperscript{T} and its phylogenetic relationship to \textit{R. tumonense} CCBAU 85046\textsuperscript{T} (Fig. 3). Furthermore, whole-genome sequences of strain 1078\textsuperscript{T} and related \textit{Rhizobium} spp. were compared by using ANI-Blast (ANIB), ANI-MUMmer (ANIm) and \textit{in silico} DDH methods. Obtained values were far below the proposed threshold for species delineation, which ranges between 95–96% for ANI\textsuperscript{8} or is 70% for DDH\textsuperscript{13–15}, confirming the authenticity of the novel species (Table 1). The ANI, ANIm and \textit{in silico} DDH values between strain 1078\textsuperscript{T} and most closely related \textit{R. tumonense} CCBAU 85046\textsuperscript{T} were 76.17%, 84.11% and 21.3%, respectively. In case of ANIm, less than 20% of the genome was aligned for all strains used for comparison, and the alignment was assigned by the software as suspicious. However, besides other strains when it was below 15%, almost 20% (19.11%) of the genome was aligned when strain 1078\textsuperscript{T} was compared with \textit{R. tumonense} CCBAU 85046\textsuperscript{T}, which is a borderline for reliable alignment. Although evidently distantly related, \textit{R. tumonense} CCBAU 85046\textsuperscript{T} was considered as a closest known relative of novel strains isolated from blackberry, with respect to their phylogenetic, phylogenomic and genomic
relatedness. Therefore, phenotypic and chemotaxonomic characterization was performed in order to determine additional traits distinguishing these two species.

The results of phenotypic characterization of novel strains are summarized in Table 2. Unlike *R. tubonense* CCBAU 85046<sup>T</sup>, the novel strains from blackberry were able to catabolize L-Alanine and D-Gluconic acid. On the other hand, *R. tubonense* CCBAU 85046<sup>T</sup> utilized L-Lactic acid, contrary to the novel strains studied. However, many genes encoding transport and catabolism of carbon and nitrogen compounds can be plasmid-borne, and therefore, the role of phenotypic tests in taxonomy of *Rhizobium* spp. has been recently called into question<sup>20</sup>. Moreover, biochemical tests were of limited value for classification and differentiation of some *Rhizobiaceae* species as indicated by Puławska, *et al.*<sup>21</sup>.

The major cellular fatty acids of the four novel strains were: 18:1 w<sub>7c</sub> (66.11–70.93%), 19:0 cyclo w<sub>8c</sub> (8.71–12.40%), Summed feature 2 (12:0 aldehyde and/or an unknown fatty acid of equivalent chain length 10.92%), and 14:0 3OH/16:1 iso I; 5.88–6.23%) and 16:0 (4.07–5.63%) (Table S2). Comparing to four strains studied, *R. tubonense* CCBAU 85046<sup>T</sup> possessed a lower content of fatty acid 18:1 w<sub>7c</sub> (55.11%), and a higher one of 16:0 (10.65%) and 11 methyl 18:1 w<sub>7c</sub> (6.72%) (Table S2).

By using PCR, presence of *virC*, *virD2*, *ipt* and *tms2* genes was detected in all four strains studied, indicating that they carry the Ti plasmid required for plant tumorigenic ability. In pathogenicity assay, all strains caused tumors on inoculated sunflower seedlings and kalanchoe plants. In contrast to strains 1078<sup>T</sup> and 1081 which clearly induced tumors on kalanchoe stems, tumors induced by strains 932 and 1019 were smaller, which could suggest differences in the virulence of the strains. In case of tomato, the reaction of plants was variable, since strains caused either very small and inconspicuous tumors, or symptom development was absent.

Overall, based on the polyphasic characterization of the four strains isolated from cane gall tumors on thornless blackberry, we propose that they represent a novel species, *Rhizobium tumorigenes* sp. nov., with 1078<sup>T</sup>.
Bacterial strains and DNA extraction. Four strains (=DSM 104880 = CFBP 8567) as the type strain. \textit{R. tumorigenes} sp. nov. is a new plant tumorigenic species containing the Ti plasmid and the second tumorigenic species within the genus \textit{Rhizobium}. Tumor-inducing ability has been limited so far to \textit{Agrobacterium} spp., \textit{A. vitis} and \textit{R. rhizogenes}.

The new species is registered at Digital Protologue website the (http://imedea.uib-csic.es/dprotologue/) under the taxonomer TA00285. The description of the new species is given in Table 2.

Materials and Methods

Table 1. Average nucleotide identity (ANI) and in silico DNA–DNA hybridization (DDH) comparisons between \textit{Rhizobium tumorigenes} sp. nov. 1078 and related \textit{Rhizobium} spp. Accession numbers refer to draft genomes or chromosome sequences.ANI-Blast. "ANI-MUMmer. Less than 20% of the genome has been aligned.

PCR melting profile (PCR MP) fingerprinting. Genetic diversity among four novel strains was investigated by a method of PCR melting profile (PCR MP) with two sets of restriction enzymes, adaptors and primers: \textit{Apa} and \textit{HindIII} as described by Puławska, et al. Denaturation temperatures 91 °C and 89 °C were used for PCR MP with \textit{Apa} and \textit{HindIII} enzymes, respectively.

PCR amplification and sequencing of 16S rRNA and housekeeping genes. The amplification and sequencing of nearly complete 16S rRNA gene was performed by using \textit{fD1} and \textit{rP2} primers, as described by Kuzmanović, et al. Primer sets atpD-273F/771R and rpoB-456F/1061R were used for amplification and sequencing of \textit{atpD} and \textit{rpoB} gene fragments, respectively. PCR reactions were performed in a 25 µl volume with master mix containing 1 × Colourless GoTaq Flexi buffer (Promega Corp., USA), 1.5 mmol l$^{-1}$ MgCl$_2$, 0.2 mmol l$^{-1}$ of each dNTP, 0.2 µmol l$^{-1}$ of each primer, 0.5 U of GoTaq Flexi DNA polymerase (Promega Corp., USA) and 40–60 ng of DNA template. The thermal profile for amplification of \textit{atpD} gene fragment was as described by Gaunt, et al., except that total of 35 cycles with annealing temperature of 60 °C, followed by final extension at 72 °C for 5 min were used. For amplification of \textit{rpoB} gene fragment, the PCR conditions were as follows: initial denaturation at 95 °C for 5 min; 35 cycles of denaturation at 94 °C for 1 min, annealing at 58 °C for 1 min and extension at 72 °C for 1 min. A final extension at 72 °C for 5 min was conducted. The amplification and sequencing of \textit{recA} gene fragment was performed by using primers F2898/F2899, as described before.

Gene sequence comparison and phylogenetic analysis. The phylogenetic analysis and sequence comparisons were conducted on 16S rRNA gene sequence, and sequences of \textit{atpD}, \textit{recA} and \textit{rpoB} housekeeping genes. Sequences of related \textit{Rhizobiaceae} strains were retrieved from NCBI GenBank and included into the analysis (Table S3). The obtained sequences were aligned using MUSCLE at EMBL-EBI. Pairwise nucleotide identities were calculated using the p-distance model with MEGA 7.0.21 software package. Maximum likelihood (ML) trees were generated with PhyML 3.0 by using 1000 bootstrap replicates. The most suitable substitution models were determined by the Smart Model Selection (SMS) tool and jModelTest 2.1.10, according to the Akaike information criterion (AIC).

| Species: | Strain: | Accession Numbers | ANI values (%) | in silico DDH (%) |
|---------|--------|------------------|----------------|-----------------|
| \textit{Rhizobium tubonense} | CCB AU 85046 | PCDP01 | 76.15 | 84.03 | 21.3 |
| \textit{Rhizobium rhizogenes} | ATCC 11325 | BAYX01 | 75.7 | 83.87 | 21 |
| \textit{Rhizobium rhizogenes} | K84 | CP000628, CP000629 | 75.65 | 83.76 | 20.9 |
| \textit{Rhizobium tropici} | CIAT 899 | CP004015 | 75.53 | 83.76 | 21 |
| \textit{Rhizobium freeii} | PRF 81 | AQHN01 | 75.24 | 83.71 | 21.1 |
| \textit{Rhizobium leucense} | USDA 9039 | AUF B01 | 75.24 | 83.76 | 21 |
| \textit{Rhizobium multihospitium} | CCB AU 83401 | FMAG01 | 75.16 | 83.72 | 20.7 |
| \textit{Rhizobium hainanense} | J66 | EMAC01 | 75.06 | 83.69 | 20.8 |
| \textit{Rhizobium eucadorense} | CNPSo 671 | LFIO01 | 75.04 | 83.94 | 20.7 |
| \textit{Rhizobium leguminosarum} | USDA 2370 | MRDL01 | 74.91 | 83.85 | 20.7 |
| \textit{Rhizobium eti} | CFN 42 | CP000133 | 74.8 | 83.82 | 20.6 |
| \textit{Rhizobium aethiopicum} | HBR26 | FMAJ01 | 74.68 | 83.62 | 20.5 |
Genome sequencing. DNA fragmentation was performed on Covaris E210 and libraries were made with NEBNext DNA Library Prep Master Mix Set for Illumina® (NEB, USA). Sequencing was performed on Illumina MiSeq platform using MiSeq Reagent Kit v2 (500-cycles) in PE250 mode generating 3,336,198 (1078T) and 3,784,696 (R. tubonense CCBAU 85046T) sequences in pairs (Genomed SA, Poland). Sequence processing and assembly were performed using CLC Genomics Workbench 7.5.

Whole-genome sequence comparisons and phylogenomic analysis. Genome sequence of strain 1078T was compared with genome sequences of related Rhizobium spp., by calculating average nucleotide identity (ANI) values using the JSpecies Web Service 35. In silico DNA–DNA hybridizations (DDH) values by the Genome-to-GenomeDistance Calculator (GGDC 2.1; http://ggdc.dsmz.de/distcalc2.php) using the recommended BLAST + alignment and formula 2 (identities/HSP length) 17 were also obtained.

Genome-wide phylogeny based on 385 conserved protein sequences extracted from genome sequences of 1078T and strains of related Rhizobiaceae strains was reconstructed by using PhyloPhlAn pipeline, version 0.99 36.
Phenotypic characterization. Novel strains isolated from blackberry, including R. tubonense CCAU 85046 were phenotypically characterized by using API and Biolog tests. The API 20NE kit was used according to manufacturer's instructions (bioMérieux) and addition of MgSO4 in order to improve bacterial growth as described before by Saidi, et al. Utilization of sole carbon sources was tested with Biolog GEN III microplates by using protocol C2 according to the instructions of the manufacturer (Biolog, Inc., Hayward, CA, USA). Measurements were taken after incubation of API strips and Biolog microplates at 20 °C for 72 h.

Chemotaxonomic analysis. Analysis of cellular fatty acid composition of the novel strains isolated from blackberry, including R. tubonense CCAU 85046 was performed by the Microbial Identification System (Sherlock version 6.1, TSBA40 method), as recommended by the manufacturer. Since the bacteria did not grow well on standard trypticase soy agar (TSA) medium, they were cultured on YMA at 22 °C for 36 h.

Detection of tumor-inducing (Ti) plasmid and pathogenicity assay. Bacterial strains isolated from blackberry were subjected to PCR analysis using primers specific for tumor-inducing (Ti) plasmid genes: virC (VGF3/VCR3)31, virD2 (A/C) and ipt (CYT1/CYT)32, and tms2 (tms2F1/tms2R2)33, as described before34.

Pathogenicity of the novel strains originating from Serbia was studied by inoculating stem internodes of young tomato (Solanum lycopersicum) and kalanchoe (Kalanchoe daigremontiana) plants, and hypocotyls of sunflower (Helianthus annuus) seedlings, as described before35.

Accession numbers. The DDBJ/EMBL/GenBank accession numbers for the partial 16S rRNA gene sequences of the strains 1081, 10787, 1019 and 932 are MG018988-MG018991, respectively. Accession numbers for the partial atpD gene sequences of the strains R. tubonense CCAU 85046, 1019, 10787, 1081 and 932 are MG007662-MG007666, respectively. Accession numbers for the partial recA gene sequences of the strains of R. tubonense CCAU 85046, 1019, 10787, 1081 and 932 are MG007667-MG007671, respectively. Accession numbers for the partial rpoB gene sequences of the strains R. tubonense CCAU 85046, 1019, 10787, 1081 and 932 are MG007672-MG007676, respectively.

The draft genome sequences of R. tumorigenes 10787 and R. tubonense CCAU 85046 have been deposited at DDBJ/EMBL/GenBank under the accession numbers PCDQ00000000 and PCDP00000000, respectively.

References
1. Otten, L., Burr, T. & Szegedi, E. In Agrobacterium: From Biology to Biotechnology (eds Tzvi Tzfira & Vitaly Citovsky) Ch. 1, 1–46 (Springer 2008).
2. Escobar, M. A. & Dandekar, A. M. Agrobacterium tumefaciens as an agent of disease. Trends Plant Sci. 8, 380–386, https://doi.org/10.1016/S1360-1385(03)00162-6 (2003).
3. Pulawska, J. Crown gall of stone fruits and nuts, economic significance and diversity of its causal agents: tumorigenic Agrobacterium spp. J. Plant Pathol. 92, S1, S7–S81.98 (2010).
4. Weller, S. A., Stead, D. E. & Mazzucchi, U. Crown and cane gall of a blackberry-raspberry hybrid caused by Agrobacterium rhizogenes. Trends Plant Sci. 8, 161–165 (2003).
5. Burt, T. J. & et al. Failure of Agrobacterium radiobacter Strain K-84 to control crown gall on raspberry. HortScience 28, 107–109 (1993).
6. Hobolth, L. A. Agrobacterium radiobacter var. tumefaciens biotype 2 found on Rubus insularis in Denmark, Botanisk-Tidsskrif 68, 160–164 (1973).
7. Peluso, R., Raio, A., Morra, F. & Zoina, A. Physiological, Biochemical and Molecular Analyses of an Italian Collection of Agrobacterium tumefaciens strains. Eur. J. Plant Pathol. 109, 291–300, https://doi.org/10.1023/A:1023556108085 (2003).
8. Sule, S. Biotypes of Agrobacterium tumefaciens in Hungary. J. Appl. Bacteriol. 44, 207–213, https://doi.org/10.1111/j.1365-2672.1978.tb00792.x (1978).
35. Richter, M., Rosselló-Móra, R., Oliver Glöckner, F. & Peplies, J. JSpeciesWS: a web server for prokaryotic species circumscription (including former Sinorhizobium). Int. J. Syst. Evol. Microbiol., 51, 2037–2048, https://doi.org/10.1099/00207713-51-6-2037 (2001).

36. Martens, M. et al. Advantages of multilocus sequence analysis for taxonomic studies: a case study using 10 housekeeping genes in the genus Ensifer (including former Sinorhizobium). Int. J. Syst. Evol. Microbiol., 58, 200–214, https://doi.org/10.1099/ijs.0.65392-0 (2008).

37. Shams, M., Vial, L., Chapulliot, D., Nesme, X. & Lavire, C. Rapid and accurate species and genomic species identification and exhaustive population diversity assessment of Agrobacterium spp. using recA-based PCR. Syst. Appl. Microbiol., 36, 351–358, https://doi.org/10.1016/j.syapm.2013.03.002 (2013).

38. Edgar, R. C. Muscle: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res., 32, 1792–1797, https://doi.org/10.1093/nar/gkh340 (2004).

39. Puławska, J. & Sobiczewski, P. Development of a semi-nested PCR based method for sensitive detection of tumorigenic Agrobacterium strains. J. Gen. Plant Pathol., 81, 37–38, https://doi.org/10.1007/s12352-014-0113-8 (2014).

40. Kuzmanović, N. PhyloPhlAn is a new method for improved phylogenetic and taxonomic characterization of the strains (API and Biolog). N.K. wrote the manuscript. All authors read, discussed, edited and approved the final manuscript.

Acknowledgements
This research was supported by the Georg Forster Fellowship for postdoctoral researchers from the Alexander von Humboldt-Foundation, Bonn, Germany and by National Science Centre, Poland grant No. DEC-2013/08/M/Z9/00138. We would like to thank Dr. Milan Stević and Dr. Ivana Jovičić from University of Belgrade – Faculty of Agriculture (Belgrade, Serbia) for providing plant samples of Rubus sp. We are grateful to the following colleagues from Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, for support in phenotypic tests and cellular fatty acid analysis: Iljana Schroeder, Gabriele Pötter, Dr. Rüdiger Pukall and Dr. Ewelina Atasayar. We thank Dr. Wen Feng Chen (China Agricultural University, Beijing, China) for kindly providing type strain of Rhizobium tubenense CCBAU 85046. The authors gratefully acknowledge Prof. Aharon Oren (The Hebrew University of Jerusalem, Jerusalem, Israel), Prof. Bernhard Schink (University of Konstanz, Konstanz, Germany) and Prof. George M. Garrity (Michigan State University, East Lansing, MI, USA) for their valuable help on nomenclature aspects.

Author Contributions
N.K. and J.P. conceived, designed and performed the experiments, and analyzed data. K.S. coordinated and provided type strain of CCBAU 85046T. The authors gratefully acknowledge Prof. Aharon Dr. Ewelina Atasayar. We thank Dr. Wen Feng Chen (China Agricultural University, Beijing, China) for kindly providing type strain of Rhizobium tubenense CCBAU 85046. The authors gratefully acknowledge Prof. Aharon Oren (The Hebrew University of Jerusalem, Jerusalem, Israel), Prof. Bernhard Schink (University of Konstanz, Konstanz, Germany) and Prof. George M. Garrity (Michigan State University, East Lansing, MI, USA) for their valuable help on nomenclature aspects.

Additional Information
Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-018-27485-z.

Competing Interests: The authors declare no competing interests.

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.