Multilocus sequence analysis supports the taxonomic position of *Astragalus glycyphyllos* symbionts based on DNA–DNA hybridization

Sebastian Gnat, Wanda Małek, Ewa Oleńska, Sylwia Wdowiak-Wróbel, Michał Kalita, Jerzy Rogalski and Magdalena Wojcik

1Department of Veterinary Microbiology, University of Life Sciences, 13 Akademicka st., 20-950 Lublin, Poland
2Department of Genetics and Microbiology, University of Maria Curie-Sklodowska, 19 Akademicka st., 20-033 Lublin, Poland
3Department of Genetics and Evolution, University of Bialystok, 1J Ciolkowskiego st., 15-245 Bialystok, Poland
4Department of Biochemistry, University of Maria Curie-Sklodowska, 19 Akademicka st., 20-033 Lublin, Poland

In this study, the phylogenetic relationship and taxonomic status of six strains, representing different phenons and genomic groups of *Astragalus glycyphyllos* symbionts, originating from Poland, were established by comparative analysis of five concatenated housekeeping gene sequences (*atpD*, *dnaK*, *glnA*, *recA* and *rpoB*), DNA–DNA hybridization and total DNA G+C content. Maximum-likelihood phylogenetic analysis of combined *atpD*, *dnaK*, *glnA*, *recA* and *rpoB* sequence data placed the studied bacteria into the clade comprising the genus *Mesorhizobium*. In the core gene phylograms, four *A. glycyphyllos* nodule isolates (AG1, AG7, AG15 and AG27) formed a cluster common with *Mesorhizobium ciceri*, whereas the two other *A. glycyphyllos* symbionts (AG17 and AG22) were grouped together with *Mesorhizobium amorphae* and *M. septentrionale*. The species position of the studied bacteria was clarified by DNA–DNA hybridization. The DNA–DNA relatedness between isolates AG1, AG7, AG15 and AG27 and reference strain *M. ciceri* USDA 3383T was 76.4–84.2 %, and all these *A. glycyphyllos* nodulators were defined as members of the genomospecies *M. ciceri*. DNA–DNA relatedness for isolates AG17 and AG22 and the reference strain *M. amorphae* ICMP 15022T was 77.5 and 80.1 %, respectively. We propose that the nodule isolates AG17 and AG22 belong to the genomic species *M. amorphae*. Additionally, it was found that the total DNA G+C content of the six test *A. glycyphyllos* symbionts was 59.4–62.1 mol%, within the range for species of the genus *Mesorhizobium*.

**INTRODUCTION**

Rhizobia are soil-inhabiting bacteria that are able to form nitrogen-fixing nodules on the roots of leguminous plants (Maróti & Kondorosi, 2014). Until 1982, all rhizobia were placed in a single genus, *Rhizobium*. Since then, the classification of rhizobia has been modified many times and, presently, 98 species of these bacteria, isolated from a wide spectrum of host plants and geographical areas, are affiliated to 12 genera of the class *Alphaproteobacteria*, *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*, *Ensifer*, *Methyllobacter*, *Devesia*, *Ochrobactrum*, *Blastobacter*, *Devosia*, *Methylobacter*.
Microvirga, Shinella and Phyllobacterium, and to two genera of the class Betaproteobacteria, Burkholderia and Cupriavidus (Berrada & Fikri-Benbrahim, 2014; Rivas et al., 2009; Willems, 2006; http://rhizobia.co.nz/taxonomy/rhizobia.html).

Nowadays, bacterial taxonomy is based on a polyphasic approach, including both phenotypic and genomic characteristics, with 16S rRNA gene sequence analysis and DNA–DNA hybridization data serving as ‘gold standards’ for descriptions of bacterial genera and species, respectively (Coenye et al., 2005; Gevers et al., 2005, 2006; Rosselló-Mora & Amman, 2001; Stackebrandt & Ebers, 2006; Stackebrandt & Liesack, 1993; Stackebrandt et al., 2002; Yarza et al., 2014). Empirically, it has been established (based on reliable, defined bacterial species) that DNA–DNA relatedness of 70 % or more, determined by hybridization, is a reasonable border for species circumscription. Although the 70 % DNA–DNA reassociation parameter is universally applicable for bacterial species designation, the DNA–DNA hybridization technique has been criticized as laborious and time consuming. Moreover, the diverse available DNA–DNA hybridization methods can yield different results, especially for lower hybridization values (Grimonet et al., 1980; Huss et al., 1983). Additionally, these techniques, due to their comparative nature, do not provide cumulative DNA–DNA relatedness values for databases (Gevers et al., 2005; Rosselló-Mora & Stackebrandt, 2006).

To establish an absolute genomic boundary for the circumscription of species, a novel tool, based on whole-genome sequences, was developed (Konstantinidis & Tiedje, 2005a, b). It was found that the 70 % DNA–DNA hybridization gold standard corresponds closely to ~94 % average nucleotide identity (ANI) of the total DNA of two strains belonging to the same species (Konstantinidis & Tiedje, 2005b). Although the ANI value is a robust and sensitive criterion for establishing the genomic relatedness between bacteria from strains to the genus level, the small number of whole-genome sequences currently available is a serious limitation for the implementation of this parameter in bacterial taxonomy (Konstantinidis & Tiedje, 2005a).

A stable framework for the estimation of the relatedness of bacteria above the species level, and tracing their phylogeny, is provided by 16S rRNA gene sequence analysis. The genus is defined as a taxon that comprises bacteria with at least 94.5 % 16S rRNA gene sequence identity (Yarza et al., 2014). It was also stated that the small-subunit (SSU) rRNA gene sequence similarity threshold of 98.7–99 % is a point at which DNA–DNA hybridization should be mandatory for testing the genomic taxonomic uniqueness of novel isolates (Stackebrandt & Ebers, 2006). The use of the 16S rRNA gene as the sole phylogenetic marker is currently being questioned, because of its presence in multiple copies within the genomes of some bacteria, evidence for intra- and intergenomic transfer of the 16S rRNA gene (including in rhizobia) and evidence for chimeric SSU rRNA gene sequences (Broughton, 2003; Eardly et al., 1996; Rajendhran & Gunasekaran, 2011).

To overcome the aforementioned drawbacks, genes encoding housekeeping proteins have been proposed as alternative phylogenetic markers to elucidate genomic relatedness at the inter- and intraspecies levels (Gevers & Coenye, 2007; Hanage et al., 2006). Sequence analysis of five to seven housekeeping genes (multilocus sequence analysis; MLSA), universally distributed among bacteria, present in single copies and dispersed in the genome, appears to be a promising approach to establish the phylogeny and taxonomic position of bacteria at the species level (Gevers et al., 2005; Hanage et al., 2006; Pérez-Yépez et al., 2014; Youseif et al., 2014). To validate a concatenated, multigene sequence analysis for species designation, it is necessary to demarcate the genomic boundary of the taxon using DNA–DNA hybridization as a reference point.

Numerical boundaries for bacterial species and genus circumscription also supply information on the G + C content of the DNA (Stackebrandt & Liesack, 1993). Empirically, it has been established that bacteria that differ by more than 10 mol% G + C in the total DNA do not belong to the same genus, and those with differences in the DNA G + C content greater than 5 mol% represent different species. The base composition criterion can only be used negatively.

The aim of this study was to evaluate the potential of MLSA of five housekeeping genes for the elucidation of the taxonomic position of symbionts of Astragalus glycyphyllos (liquorice milkvetch) at the species level, by comparing these data with those from DNA–DNA hybridization, to affiliate A. glycyphyllos nodulators into species and to estimate the DNA G + C contents of these microorganisms, which is useful for separating bacterial genera and species. It is worth noting that, presently, limited information is available on bacteria that form nitrogen-fixing associations with the ecologically important leguminous plant A. glycyphyllos, which serves as a green manure, a soil coverage reducing erosion and as a herbal medicine.

METHODS

Bacterial strains and growth conditions. For MLSA, DNA–DNA hybridization and analysis of DNA G + C content, A. glycyphyllos nodule isolates AG1, AG7, AG15, AG17, AG22 and AG27 were used (Gnat et al., 2014). Additionally, for the determination of DNA–DNA hybridization by a microplate method, Mesorhizobium amorphae ICMP 15022T, M. ciceri USDA 3383T, M. septentriionale SDW014T, M. temperatum LMG 23931T, M. loti USDA 3471T, M. albiziae CCB01 61158T and M. huakuii USDA 4779 were used as reference strains. Bacterial cultures were grown at 28 °C in yeast extract-manitol (YEM) medium and kept at 4 °C (Vincent, 1970).

DNA isolation. Total DNA was extracted from each strain using the method of Pitcher et al. (1989). The purity and concentration of DNA were measured using a spectrophotometer (Bio-Rad SmartSpec 3000).

http://ijs.microbiologyresearch.org
Amplification and sequencing of atpD, dnaK, glnA, recA and rpoB genes. To clarify the phylogenetic relationship of A. glycyphyllos nodulators and establish their species position, strains AG1, AG7, AG15, AG17, AG22 and AG27, representing different bacterial clusters identified by numerical taxonomy (Gnat et al., 2014), were used for sequence analysis of five housekeeping genes. All PCR amplifications were performed in 25 μl PCR mixtures (Ready-Mix Taq Tag, Sigma) containing 50 ng DNA and 30 pmol of each primer using a Perkin-Elmer model 2400 thermocycler. atpD sequences were amplified and sequenced with the forward primer atpDF1 (5′-ATCGGCGCCGCTGACAGA-3′) and reverse primer atpDr (5′-GGCGAGCCGGTCGACGA-3′) (Gaunt et al., 1997). The PCR mixture was incubated at 95 °C for 2.5 min, followed by 30 rounds of thermal cycling (94 °C for 45 s, 50 °C for 60 s and 72 °C for 90 s) and a final elongation step at 72 °C for 7 min. The 3′ regions of the dnaK gene were amplified and sequenced with primer TsdnaK4 (5′-GTACATGGCCCTCGGAGGTTCA-3′) and TsdnaK2 (5′-GGGAGAAGGCCCAGGAAAGG-3′) corresponding to positions 1057–1075 and 1794–1772, respectively, of the dnaK gene of Bradyrhizobium japonicum USDA 110 (Stepkowski et al., 2003). The PCR conditions for amplification were as follows: initial denaturation at 94 °C for 90 s followed by 35 cycles of 60 s a 94 °C, 60 s at 55 °C and 40 s at 72 °C and a final extension of 7 min at 72 °C. For amplification of internal fragments of the glnA gene, forward primer glnA144f (5′-GTCATGTTTTCAGGAGAACGCA-3′) and reverse primer glnA1142r (5′-TGGAACTCGTGCTCTCG-3′) were used (Martens et al., 2008). The conditions for PCR amplification were as follows: 10 min at 95 °C, 2 cycles of 2 min at 94 °C, 2 min at 62 °C and 1 min at 72 °C, 32 cycles of 30 s at 94 °C, 1 min at 61 °C and 1 min at 72 °C and a final extension step at 72 °C for 10 min. recA gene fragments corresponding to positions 6–555 of the recA gene of Rhizobium leguminosarum bv. viciae 39 (GenBank accession no. X59956) were amplified with primers rec66f (5′-CGGCTCGAGTAGAGAAATC-3′) and recA555r (5′-GGTATCTGGTGAAGAATC-3′) (Martens et al., 2007) using the following protocol: initial denaturation at 95 °C for 3 min, 35 cycles of 94 °C for 45 s, 60 °C for 60 s and 74 °C for 90 s and a final extension step at 72 °C for 5 min. For amplification of the rpoB gene, forward primer rpoB83f (5′-CCATCGGTTTCCAGAGAAC-3′) and reverse primer rpoB1061r (5′-AGGCGCATGGTGGGATAGGG-3′) corresponding to positions 83–101 and 1081–1061, respectively, of the complete genome of Ensifer mellitii 1021 were used (Martens et al., 2008). The PCR was performed under the following cycling conditions: 5 min at 95 °C, three cycles of 2 min at 94 °C, 2 min at 58 °C and 1 min at 72 °C, 30 cycles of 30 s at 94 °C, 1 min at 58 °C and 1 min at 72 °C and a final elongation step at 72 °C for 5 min.

Sequence data analyses. The individual and concatenated atpD, dnaK, glnA, recA and rpoB gene sequences generated in this study were aligned with those available in the GenBank database using CLUSTAL_X and optimized manually with GeneDoc (Nicholas & Nicholas, 1997). Gene phylogenies were reconstructed using the maximum-likelihood (ML) method implemented in the PAUP software (version 4.0b10). ML analysis was performed with the PhyML version 2.4.5 software (Guindon & Gascuel, 2003) using MODELTEST 3.7 (Posada & Crandall, 1998) to choose the best-fit evolutionary model for each analysed gene. Statistical support for tree nodes was evaluated by bootstrap analysis with 100 samplings. The phylograms were visualized with TreeView (Page, 1996).

DNA–DNA hybridization and quantitative detection of DNA–DNA hybrids. Bacterial genomic DNA was prepared according to the method of Pitcher et al. (1989). Colorimetric DNA–DNA hybridization was carried out using a microplate method in which unlabelled DNA, bound non-covalently to a CovaLink microplate (Nunc), was hybridized with biotin-labelled DNA for 18–20 h at 45 °C (Ezaki et al., 1989; Kalita et al., 2004). Streptavidin–peroxidase conjugates were used to detect DNA–DNA hybrids by measurement of their absorbance at 450 nm with a microplate reader (Bio-Rad model 550) (Kalita et al., 2004). The rate of DNA–DNA hybridization was calculated according to Ezaki et al. (1989).

RESULTS AND DISCUSSION

In our earlier studies, 28 A. glycyphyllos nodule isolates were classified into the genus Mesorhizobium, based on numerical analysis of phenotypic properties, almost full-length 16S rRNA gene sequences and 16S rRNA gene-based RFLP genotyping (Gnat et al., 2014). The SSU rRNA gene sequence-based phylogenetic tree of six A. glycyphyllos symbionts, representing different phyla and genotypes of these bacteria, clearly demonstrated that two of these strains, AG17 and AG22, are close phylogenetic neighbours of M. amorphae and M. septentrio nale (98–99 % sequence similarity), whereas the four other strains, AG1, AG7, AG15 and AG27, showed a close phylogenetic relationship to M. ciceri (95–99 % sequence similarity). Although 16S rRNA gene sequence analysis is a standard in bacterial taxonomy and phylogeny, its use as the sole molecular marker has been criticized, because of its low resolving power at the species level and for some genera (Janda & Abbott, 2006). To overcome these drawbacks, and to clarify the genus and species status of the liquorice milkvetch symbionts, we sequenced the following five protein-coding genes, atpD (ATP synthase F1, beta subunit), dnaK (heat-shock protein 70), glnA (glutamine synthetase), recA (recombinase A protein) and rpoB (RNA polymerase, beta subunit), of A. glycyphyllos nodules (the same isolates as used in the 16S rRNA gene sequence analysis) and compared their sequences with those of reference rhizobia, representing different genera and species in the order Rhizobiales, available in the GenBank database. The choice of genes was guided by the following requirements: universal presence, single-copy occurrence, a relatively high level of conservation and wide distribution on the chromosome (Gevers & Coenye, 2007; Martens et al., 2007; Stackebrandt et al., 2002). ML phylogenies were inferred for each of the five housekeeping loci separately (Figs S1–S5, available in the online Supplementary Material) and for the concatenated atpD, dnaK, glnA, recA and rpoB gene sequences (Fig. 1). In all individual gene trees (Figs S1–S5), the A. glycyphyllos nodulators were consistently clustered with species of the genus Mesorhizobium (at 90–99 % similarity), in agreement with their grouping based on 16S rRNA gene sequences (Gnat et al., 2014). The species of Ensifer (Sinorhizobium) plus Rhizobium formed a well-supported sister clade to the monophyletic cluster formed by Mesorhizobium, whereas the species of the genus Bradyrhizobium and...
Azorhizobium caulinariondans constituted a distinct lineage(s) on the outskirts of the phylograms. Four liquorice milk-vetch nodulators, AG1, AG7, AG15 and AG27, together with strains of M. ciceri, the symbiont of Cicer arietinum (Nour et al., 1995) and M. loti (except for the atpD gene tree), the symbiont of species of the genus Lotus (Jarvis et al., 1997), occupied an independent branch (79–96% bootstrap support). Two other strains, AG17 and AG22, formed a tight cluster (81–97% bootstrap support) along with strains of M. septentrionale, the microsymbiont of Astragalus adsurgens (Gao et al., 2004), and M. amorphae, a microsymbiont of Amorpha fruticosa (Wang et al., 1999), except for the recA gene tree, where strain AG22 clustered together with strains AG1, AG7, AG15 and AG27 but not with isolate AG17 (Fig. S5). A new position of the A. glycyphyllos symbiont AG22 in the recA gene phylogram might be the result of horizontal transfer of this gene between bacteria of the genus Mesorhizobium, as well as different recombination and mutation rates of the recA gene in comparison with the other studied core genes. The recA gene phylogram (Fig. S5) leads to the conclusion that a single-gene tree may inadequately reflect the phylogenetic relationship of bacteria. The ad hoc committee for the re-evaluation of the species definition in bacteriology proposed the analysis of a minimum of five housekeeping genes to achieve an adequate, informative level of phylogenetic data (Stackebrandt et al., 2002). MLSA is now a quite common practice in taxonomic studies. This technique was used in this study, based on five protein-coding genes, to elucidate the taxonomic position of A. glycyphyllos symbionts in the genus Mesorhizobium. The alignment of the combined sequences of the housekeeping genes of A. glycyphyllos symbionts and reference strains, representing the genera Mesorhizobium, Ensifer, Rhizobium and Bradyrhizobium, contained a total of 3030 positions, of which 1996 were constant, 241 variable but parsimony uninformative, and 793 were parsimony informative. A phylogram of the five concatenated housekeeping gene sequences, reconstructed by the ML method, is shown in Fig. 1. There is strong agreement in the placement of the test A. glycyphyllos rhizobia, with respect to reference rhizobia, in the phylogram based on the five concatenated sequences and those based on the individual atpD, dnaK, glnA, recA and rpoB (Figs S1–S5) and 16S rRNA (Gnat et al., 2014) gene sequences. It is also worth underlining that, in the concatenated gene tree (Fig. 1), the bacterial groups were supported by higher bootstrap values than in the single gene trees (Figs S1–S5), which means they are more robust and more reliable. In a phylogram of the combined sequences, the test rhizobia were in a clade formed by species of the genus Mesorhizobium and formed, along with them, a highly supported monophyletic group (99% bootstrap support) with a nucleotide sequence similarity level of 92–97%. The A. glycyphyllos symbionts clustered into two distinct subgroups (Fig. 1), as in the atpD, dnaK, glnA, recA (except for strain AG22) and rpoB single-gene trees (Figs S1–S5). Two representatives of the A. glycyphyllos symbionts, AG17 and AG22, were tightly grouped with the type strains of M. amorphae and M. septentrionale (100% bootstrap support), whereas the remaining studied nodule isolates, AG1, AG7, AG15 and AG27, were close phylogenetic neighbours of the type strain of M. ciceri, and all these strains together formed a common cluster, at bootstrap support of 99%. Based on the data from our combined sequence analysis, the A. glycyphyllos nodulators AG1, AG7, AG15 and AG27 may be affiliated to the genomospecies M. ciceri, but it remains unclear whether nodule isolates AG17 and AG22 belong to the species M. amorphae or represent M. septentrionale. We also suppose that the conflicting phylogenetic signals from the recA gene sequence concerning strain AG22 (Fig. S5) were levelled out not only in the phylogram of all concatenated genes (Fig. 1) but also in the phylograms of combined atpD+recA, atpD+dnaK+recA, atpD+dnaK+glnA+recA, dnaK+recA, glnA+recA and rpoB+recA sequences (data not shown). In all these trees with two, three, four or five combined genes sequences, isolate AG22 was located in a common cluster with AG17 and strains of M. amorphae and M. septentrionale. To explain conclusively the allocation of the A. glycyphyllos symbionts into species of the genus Mesorhizobium, established by analysis of five combined housekeeping gene sequences, the same set of studied nodule isolates was used to measure their overall DNA relatedness to DNA of reference mesorhizobia that are the closest phylogenetic
relatives of the liquorice milkvetch symbionts (Fig. 1) (Gnat et al., 2014). Presently, DNA–DNA hybridization is a cornerstone of genome characterization for the validation of the species status of bacteria (Gevers et al., 2005; Rosselló-Mora & Amann, 2001; Stackebrandt et al., 2002), and 70 % DNA–DNA relatedness constitutes the boundary to delineate bacterial species. The studied milkvetch nodulators were located in two distinct genomospecies, on the basis of DNA–DNA hybridization data. Strains AG17 and AG22 and M. amorphae ICMP 15022T exhibited 77.5–80.1 % DNA–DNA relatedness, indicating that strains AG17 and AG22 belong to the genomic species M. amorphae. The DNA–DNA relatedness of nodule isolates AG17 and AG22 to other reference mesorhizobia was far below the species limit, i.e. 21.6–49.5 %, except with M. septentrionale SDW014, with which it ranged from 51.7 to 53.8 % (Table 1). The remaining A. glycyphyllos nodule bacteria analysed (AG1, AG7, AG15 and AG27) revealed levels of DNA–DNA relatedness above the species limit with M. ciceri USDA 3383 (76.4–84.2 %) and much lower DNA–DNA relatedness to the other reference mesorhizobia (17.9–50.1 %) (Table 1), supporting the affiliation of these liquorice milkvetch rhizobia to the species M. ciceri. The DNA–DNA reassociation rates of the studied A. glycyphyllos symbionts representing the genomic species M. amorphae and M. ciceri also supported their different species allocation. The hybridization values of strains AG17 and AG22 with DNA of isolates AG1, AG7, AG15 and AG27 ranged from 43.5 to 51.0 %, and were much higher within the genomospecies M. amorphae and M. ciceri, i.e. 92.8 and 89.5–95.5 %, respectively (data not shown).

Our DNA–DNA reassociation results are generally coherent with the combined housekeeping gene sequence data and confirmed the assumption drawn from MLSA, that the A. glycyphyllos symbionts AG1, AG7, AG15 and AG27 belong to the genomic species M. ciceri, whereas the nodule isolates AG17 and AG22 are certainly members of the genomospecies M. amorphae. The ad hoc committee for the re-evaluation of the species definition in bacteriology (Stackebrandt et al., 2002) recommended the analysis of a minimum of five protein-coding gene sequences in order to circumscribe a bacterial species. A year later, Zeigler (2003) found that a smaller number of genes, or even a single, carefully selected core gene, can equal or even surpass the precision of DNA–DNA reassociation for the quantification of genome relatedness and assigning related bacteria to the correct species. The taxonomic potential of the MLSA technique, confirmed by DNA–DNA relatedness, has allowed the assignment of many nodule bacteria into species (Martens et al., 2008; Merabet et al., 2010; Rincón-Rosales et al., 2009; Vinuesa et al., 2005). The MLSA technique has also been applied for rapid and reliable species identification in other bacterial genera, e.g. Enterococcus, Aeromonas and Haemophilus (Naser et al., 2005; Norskov-Lauritsen et al., 2004).

<table>
<thead>
<tr>
<th>Table 1. DNA–DNA relatedness between A. glycyphyllos symbionts and reference type strains of the genus Mesorhizobium</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA–DNA relatedness (%) with</td>
</tr>
<tr>
<td>Strain</td>
</tr>
<tr>
<td>AG1</td>
</tr>
<tr>
<td>AG7</td>
</tr>
<tr>
<td>AG15</td>
</tr>
<tr>
<td>AG17</td>
</tr>
<tr>
<td>AG22</td>
</tr>
<tr>
<td>AG27</td>
</tr>
</tbody>
</table>

DNA–DNA hybridization values are means of three independent experiments. In each experiment, all hybridization reactions were done in quadruplicate.
A genomic feature that is used widely in bacterial taxonomy is the DNA base composition, expressed in mol% G+C. Initially, information on the DNA base composition served to distinguish between phenotypically similar and genomically different strains (Stackebrandt & Liesack, 1993). We used the analysis of DNA G+C content (based on the HPLC method) to support the classification of the A. glycyphyllos symbionts into the genus Mesorhizobium, earlier documented by comparative 16S rRNA gene sequence analysis (Gnat et al., 2014) and, next, by MLSA of five housekeeping genes (Fig. 1). The G+C content of the genomic DNA of the six studied bacteria ranged from 59.4 to 62.1 mol% (Table 1). These values are similar to those reported for recognized species of Mesorhizobium (59–64 mol%) (Jarvis et al., 1997; Mierzwa et al., 2010; Wang et al., 1999), supporting the conclusion that the liquorice milkvetch rhizobia do indeed belong to the genus Mesorhizobium. The DNA base composition was the first feature used to characterize the bacterial genome and the description of bacterial species and genera. The DNA G+C content of different prokaryotes varies widely, from 24 to 76 mol%, and it is now generally accepted that bacteria with DNA differing in its G+C content by more than 5 mol% should not be classified into the same species, whereas those with DNA differing in its G+C content by more than 10 mol% should not be affiliated to the same genus (Stackebrandt & Liesack, 1993). Today, the DNA base composition of the type strain is required in the description of the type species of a new genus and is an optional criterion in the description of a novel species in a previously recognized genus (Stackebrandt & Liesack, 1993).

In conclusion, it is necessary to underline that the taxonomy of bacteria is a dynamic field, and the major impulse for recent changes in the taxonomic scheme of these organisms was information on their phylogeny, derived from sequence analysis of the 16S rRNA gene and five combined housekeeping genes as well as information on the total DNA relationship of bacteria, based on DNA–DNA hybridization.

ACKNOWLEDGEMENTS

This research was supported by a grant from the National Science Centre Poland (2011/03/B/NZ8/02142). We thank Prof. Wen Xin Chen for sending us M. septentrionale SDW014 strain.

REFERENCES


