Diversity of *Mesorhizobium* Species Nodulating Some Wild Legumes in Samsun Province of Turkey

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Abstract: In this study, microsymbionts of two wild legumes, *Argyrolobium biebersteinii* (Ball in Feddes) and *Lotus angustissimus* L., collected from Samsun province of Turkey were investigated using conventional and molecular methods. A total of five rhizobial isolates which produced active root nodules on their original hosts were considered. Identifications of the isolates were depending on the phylogenetic analyses of two housekeeping genes, 16S rDNA and recA. As a result of phylogenetic analyses, all isolates appeared within *Mesorhizobium* lineage. Of these isolates, OKN-1 and OKN-4 identified as *Mesorhizobium tarimensense* and OKN-3 identified as *M. japonicum*. On the other hand, other two isolates, OKN-7 and OKN-4 did not match up with any known *Mesorhizobium* species. In this study we present the first *Mesorhizobium* isolates identified using valid molecular methods from Turkey. We also give the first reports of *M. tarimensense* and *M. japonicum* from Turkey and Europe. Additionally, the new *M. tarimensense* and *M. japonicum* isolates in this study are the first isolates reported after the description of these two species from their original hosts and locations. In this study we also present molecular evidences for two new *Mesorhizobium* species, but this presumption needs further investigations.

Keywords: 16S rDNA, *Mesorhizobium*, phylogeny, recA, wild legumes.

Türkiye’nin Samsun İlindeki Bazı Yabani Baklagıl Türlerini Nodüle Eden *Mesorhizobium* Türlerinin Çeşitliliği

Oz: Bu çalışmada Türkiye’nin Samsun ilinden toplanan iki yabancı Baklagıl türünün (*Argyrolobium biebersteinii* (Ball in Feddes) ve *Lotus angustissimus* L.) mikrosimbiyontları geleneksel ve moleküler yöntemler kullanılarak incelenmiştir. Toplamba orijinal konakçılardan aktif kök nodüller oluşturan beş adet ritobiyyal izolat incelenmiştir. Bu izolatların tanımları 16S rDNA ve recA genine dayanarak yapılmıştır. Filogenetik analizleri sonucunda tüm izolatlar *Mesorhizobium* cinsine ait olduğu tespit edilmiştir. Bu izolatların üçü *M. tarimensense* ve iki adet *M. japonicum* türleri olarak tanımlanmıştır. Diğer iki izolat da Avrupadan veya Türkiye’den başka bir cinsine ait olan, ancak 16S rDNA ve recA genlerindeki benzerliklerle Türkiye’deki *Mesorhizobium* cinsine ait kendi cinsine ait dört izolat olarak tanımlanmıştır.

Anahtar sözcükler: 16S rDNA, *Mesorhizobium*, recA, yabancı baklagiller.
INTRODUCTION

Although 80% of earth’s atmosphere composed of nitrogen gas (N\textsubscript{2}), this form of nitrogen cannot be directly used by most of the living organisms to synthesis nitrogen containing organic compounds. Initially, nitrogen gas should be reduced to ammonia (biologically available nitrogen) by combining it with hydrogen and this process is called nitrogen fixation (Burdass, 2002). Biological reduction of nitrogen gas to ammonia, so called biological nitrogen fixation, is restricted to prokaryotic species which are distributed across both Bacteria and Archaea domains and provides about 65% of biosphere’s available nitrogen (Lodwig et al., 2003; Raymond et al., 2004). Some of these prokaryotes can only able to fix nitrogen when they are in a symbiotic associations with their particular hosts and that is why they have been named as symbiotic diazotrophs (Sawada et al., 2003). Rhizobia, one of the best known symbiotic diazotrophic microorganisms, are soil bacteria that form root or stem nodules on leguminous plants, where they can undertake symbiotic fixation of atmospheric nitrogen (Moreira et al., 1998). Until 1980s, all root nodulating symbiotic diazotrophic bacteria were classified within a single genus namely Rhizobium with six species, R. leguminosarum, R. phaseoli, R. trifolii, R. melliloti, R. lupini, R. japonicum, depending on specific host plant selection which was also called cross inoculation group concept (Somasegaran & Hoben, 1985). On the other hand, this concept was abandoned because it is understood that in fast-growing rhizobia, the genes coding for symbiotic associations are located on giant transmissible plasmids called symbiotic plasmids (Brewin et al., 1980). During this period, bacterial systematics underwent a huge change with the impressive contribution of molecular biology. Rhizobial systematics were also affected from this change and eventually species and genus limitations were reassessed due to polyphasic approach which considers molecular data (DNA-DNA hybridization, phylogenetic analyses depending on the nucleotide sequences of different genes) as well as phenotypic features (Graham et al., 1991). In the course of time, numbers of rhizobial species and genera have dramatically increased due to these changes in rhizobial systematics and also to the investigation of more legume species in the meaning of their symbiotic partners (Willems, 2006). Currently rhizobia are classified within 238 species (18 genera) of α- and β-Proteobacteria classes and this number is expected to increase since only 23% of legume species (roughly 19000 species exists) were investigated in the context of their symbiotic partners (Shamseldin et al., 2017). Mesorhizobium, the main objective of the study, was suggested as a new genus by Jarvis et al., (1997) depending on the differences in 16S rDNA sequences and also fatty acid profiles. Researchers classified five species, M. cicer, M. huakuii, M. loti, M. mediterraneum and M. tianhanense within this newly suggested genus. Currently, there are 41 Mesorhizobium species most of which nodulates mimosoid temperate wild legumes except M. cicer, M. mediterraneum and M. muleiense, which nodulates Cicer arietinum L (Sprent, 2007; Shamseldin et al., 2017; http://www.bacterio.net/mesorhizobium.html). From Turkey there are no records for any Mesorhizobium species identified using valid molecular methods from cultivated or wild legumes.

The main goal of the study is to identify some rhizobial samples isolated from root nodules of two wild legume species, Argyrolobium biebersteinii (Ball in Feddes) and Lotus angustissimus L., collected from Samsun province of Turkey.

MATERIAL and METHODS

In this study, rhizobial strains were isolated from root nodules of two wild Leguminous species, Argyrolobium biebersteinii (Ball in Feddes) and Lotus angustissimus L., collected from Samsun province of Turkey. Bacterial isolations from active (pink colored) root nodules were made using the method of Somasegaran and Hoben, (1985). YMA (Yeast Extract Mannitol Agar) medium was used for isolations. After 2-7 days of incubation at 26°C, typical colonies were re-inoculated on the new YMA medium for further analyses. Purity of the isolates were checked with microscopic examination by gram staining. Nodulation tests were performed using the method of Vincent (1970) with the conditions explained in Gurkanli et al. (2013). For genomic DNA isolations, isolates were grown in TY (Tryptone Yeast Extract) broth media (Ditta et al. 1987) at 28°C for 2 days. The CTAB/NaCl miniprep method (Maniatis et al. 1982; Temizkan and Arda 2004) was used for genomic DNA extractions and the DNA were stored at -20°C prior to use. Two housekeeping genes, 16S rDNA (Small subunit of rDNA) and recA (Recombinase A) were analysed for identification of rhizobial isolates. PCR amplifications of the genes were performed using a MWG Primus thermal cycler with the protocols and DNA primers shown in Table 1. A 50 µl PCR mixture for all genes were prepared as follows: 5 µl of 10X PCR buffer (Fermentas), 1.5 mM MgCl\textsubscript{2}, 0.8 mM dNTP mix (Amresco), 0.4 pmol of each primer in final concentration, 1.25 U Taq polymerase (Fermentas), template DNA<0.5 µg and ddH\textsubscript{2}O. Visualization of the PCR products which were electrophoresed on 1% agarose gel (prepared in 1X Tris-Borate-EDTA buffer and stained with ethidium bromide) were made using the GeneGenius Bio imaging system (Syngene, Synoptics Group, Cambridge, UK). Nucleotide sequencings were performed from both strands with the primers used for the PCR amplifications (Table 1). The only exception was an extra 16S rDNA internal primer pF (Zhang et al., 1999) that we used for more reliable sequencings.
The nucleotide sequencings were made commercially by Macrogen Inc. (Korea). Nucleotide sequencings from both strand were checked and assembled using BioEdit (Hall, 1999). ClustalX (Thompson et al., 1997) was employed for multiple nucleotide sequence alignments. To determine the most appropriate DNA substitution model for our data sets, the Akaike information criterion (AIC) (Akaike, 1974) and Bayesian information criterion (BIC) tests were applied with jModelTest v. 0.1 package program (Guindon & Gascuel, 2003; Posada, 2008). Initial phylogenetic analyses were performed with extended data sets comprised of 16S rDNA and recA haplotypes of all valid *Mesorhizobium* species.

![Table 1](https://example.com/1.png)  
**Table 1.** Nucleotide primers and PCR conditions used for the PCR amplifications of 16S rDNA and *recA* genes in this study.  

| Gene          | Primer ID    | D | C | A | E | FE |
|---------------|--------------|---|---|---|---|----|
| 16S rDNA      | 5′-GACAGCAGCATGATAAGATG-3′ | 3 min | 1 min | 5 min | 1.5 min | 5 min |
| recA Forward  | 5′-GACAGCAGCATGATAAGATG-3′ | 3 min | 1 min | 5 min | 1.5 min | 5 min |
| recA Reverse  | 5′-GACAGCAGCATGATAAGATG-3′ | 3 min | 1 min | 5 min | 1.5 min | 5 min |

**Table 2:** Strain informations of *Mesorhizobium* isolates obtained in this study and the type strains of *Mesorhizobium* species download from NCBI for phylogenetic analyses.

| Species          | Strain | 16S rDNA | recA |
|------------------|--------|----------|------|
| *M. tarimense*   | OKN-1  | MN647524 / This study | MN658186 / This study |
| *M. japonicum*   | OKN-3  | MN647525 / This study | MN658187 / This study |
| *M. tardigalense*| OKN-4  | MN647526 / This study | MN658188 / This study |
| *M. cicer*       | OKN-7  | MN647527 / This study | MN658189 / This study |
| *M. huakui*      | OKN-10 | MN647528 / This study | MN658190 / This study |
| *M. amorphae*    | ACCC 19665 | - | MN658191 / This study |
| *M. australicum* | WSM2073 | AY601516 / Nandasena et al., (2009) | JN202310 / Zhang et al. Unpublished |
| *M. cattuarus*   | ICMP 19515 | JNP3192 / De Meyer et al., (2015) | JK23767 / De Meyer et al., (2015) |
| *M. caraganae*   | CCBAU 11299 | EF149903 / Guan et al., (2008) | EU249394 / Guan et al., (2008) |
| *M. cicer*       | UPM-C1 | U07934 / Nour et al., (1994) | MN658191 / This study |
| *M. erdmani*     | USA3 4471 | KM192334 / Martinez-Hidalgo et al., (2015) | - |
| *M. gobiense*    | CCBAU 83330 | EF030506 / Han et al., (2008) | EF546941 / Han et al. unpublished |
| *M. helmanticense* | CSSC115 | Sannazzaro et al. unpublished | Sannazzaro et al. unpublished |
| *M. haakii*      | CCBAU 260 | D13431 / Ouyaz et al., (1993) | EU249391 / Juan et al., (2008) |
| *M. japonicum*   | MAEF03099 | NC_002674 / Martinez-Hidalgo et al., (2016) | NC_002674 / Martinez-Hidalgo et al., (2016) |
| *M. jarvisi*     | ATCC 33669 | KM192335 / Martinez-Hidalgo et al., (2015) | KM192345 / Martinez-Hidalgo et al., (2015) |
| *M. loti*        | NZP 2213 | X67229 / Willems et al., (1993) | EU033978 / Alexandre et al., (2008) |
| *M. metallidurans* | STM 2683 | AM930381 / Vital et al., (2009) | AM930382 / Vital et al., (2009) |
| *M. maleeense*   | CCBAU 83963 | - | H0116782 / Zhang et al., (2012) |
| *M. opportunism* | WSM2075 | AY601515 / Nandasena et al., (2006) | - |
| *M. pingchengii* | CCBAU 33460 | JQ339778 / Zheng et al., (2013) | JQ339757 / Zheng et al., (2013) |
| *M. septentrionale* | SDW014 | - | EF639843 / Han Unpublished |
| *M. shangrilei*  | CCBAU 65527 | EU074203 / Lu et al., (2009) | EF546948 / Han Unpublished |
| *M. tarimense*   | CCBAU 83306 | EF035058 / Han et al., (2008) | - |
| *M. tianshanense* | A-1BS | AF041447 / Wang et al., (1999) | EU249392 / Guan et al., (2008) |
| *M. waimense*    | ICMP 19557 | KC237387 / De Meyer et al., (2015) | KC237667 / De Meyer et al., (2015) |

Subsequently, more detailed analyses were conducted with concentrated data sets only composed of closely related *Mesorhizobium* species with their haplotypes (Table 2). Neighbor-Joining (NJ), Maximum-Likelihood (ML) and Maximum-Parimony (MP) analyses were performed to evaluate the phylogenetic relationships. PAUP* v. 4.0b10 (Swoford, 1998) was used for the NJ and MP analyses and PhyML 3.0 (Guindon & Gascuel, 2003) was used for ML analysis. MP analysis were performed with the heuristic search approach by using the TBR swapping algorithm (10 random repetitions). To determine the reliability of the trees, the Bootstrap tests were performed with 10000 pseudo replicates for NJ and 1000 pseudo repilicates for MP and ML. All our new 16S rDNA and recA sequences obtained in this study were deposited in GenBank under accession numbers MN647524-MN647528 and MN658186-MN658190, respectively (Table 2).

**RESULTS**

As the result of bacterial isolations, a total of 5 rhizobial samples were obtained from root nodules of *Argyrolobium biebersteinii* P.W. Ball (OKN-1, OKN-4, OKN-7, OKN-10) and *Lotus angustissimus* L. (OKN-3). The isolates formed active (pink colored) root nodules on their original hosts. To identify our isolates, we analysed two housekeeping genes, 16S rDNA and recA. We sequenced approximately 1315 bp of 16S rDNA of our isolates and BLAST (Basic Local Alignment Search Tool) results indicated that all our samples belonged to the genus *Mesorhizobium*. Phylogenetic analyses were performed with 1258 aligned nucleotides containing 76 segregating sites. AIC and BIC tests suggested GTR+I+G (I=0.838; G=0.785) substitution models, respectively. Since it gave higher bootstrap values we preferred the NJ tree drawn with TIP3uf+1 model (Figure 1). As a result, our isolates, OKN-1 and OKN-4 from *A. biebersteinii* showed the same 16S rDNA haplotype with each other and also with *M. tarimense*, *M. gobiensis*, *M. helmanticensis* and *M. metallidurans* and formed a lineage with *M. tianshanense*. Our other isolate OKN-7, also from *A. biebersteinii*, showed a unique 16S rDNA haplotype and appeared as sister to *M. caraganae* with 99.7% nucleotide sequence similarity. This
relationship was supported with 62%, 63% and 61% bootstrap values in NJ, ML and MP trees, respectively.

**Figure 1.** NJ tree based on the 16S rDNA nucleotide sequences of our new *Mesorhizobium* isolates (Bold) and the type strains of closely related *Mesorhizobium* species downloaded from GenBank (Table 2). The tree was rooted with *Sinorhizobium arboris* (Zhang et al., 1991) and *S. terangae* (de Lajudie, 1994). Bootstrap values (≥ 50%) obtained from NJ, ML and MP analyses are given on each related node.

OKN-10, our fourth isolate from *A. biebersteinii*, appeared as sister to *M. cantuariense* and *M. cicer* (showed the same 16S rDNA haplotypes) with 99.9% nucleotide sequence similarity and this node was supported with 75% and 74% bootstrap values in NJ and ML trees, respectively. On the other hand, this value was less than 50% in MP tree. *M. loti* also appeared as the fourth species in the lineage. Although this lineage was quite consistent and appeared in all trees created with NJ, ML and MP algorithms, intralineage relationships were different. In NJ and ML trees, topology were the same as explained above and all nodes in this lineage were supported with significant bootstrap values. On the other hand, a polytomy appeared in the MP bootstrap tree, thus some bootstrap values were less than 50%. Isolate OKN-3, from *L. angustissimus*, showed the same 16S rDNA haplotype with *M. japonicum* and *M. erdmanii*. *M. opportunistum* appeared as sister with 99.7% nucleotide similarity. This relationship supported with 83%, 82% and 58% bootstrap values in NJ, ML and MP trees, respectively. *M. jarvisii*, *M. huakuii* and *M. waimense* also appeared in the lineage (Figure 1).

As the second housekeeping gene we sequenced approximately 570 bp of recA gene of our isolates. Phylogenetic analyses were carried over 317 aligned nucleotides with 98 segregating sites. AIC and BIC tests were suggested TIM2+I+G (I=0.383; G=0.388) and TrN+G (G=0.172) substitution models, respectively. Since the tree created with TIM2+I+G model gave higher bootstrap values, this tree preferred in the study (Figure 2). In general, recA phylogeny was concordant with the 16S rDNA phylogeny with minor differences. Isolates OKN-1 and OKN-4 showed close recA haplotypes with *M. tarimense* with 99.6% nucleotide similarities and this relationship supported with 99%, 99% and 93% bootstrap values in the NJ, ML and MP trees, respectively. *M. gobiense*, *M. muleiense* and *M. tianshanense* also appeared in this lineage (Figure 2). Phylogenetic position of OKN-7 in recA phylogeny was quite different than 16S rDNA phylogeny. In the recA tree (Figure 2), this isolate (together with *M. caraganae*) appeared as sister to *M. metallidurans*, *M. septentrionale*, *M. waimense*, *M. amorphae* and *M. helmanticense* with 94.9%, 95.5%, 95.8%, 95.2%, 96.2% nucleotide sequence similarities, respectively. On the other hand, this lineage was not supported with enough (≥50) bootstrap values.

**Figure 2.** NJ tree based on the recA nucleotide sequences of our new *Mesorhizobium* isolates (Bold) and the type strains of closely related *Mesorhizobium* species downloaded from GenBank (Table 2). The tree was rooted with *Sinorhizobium arboris* (Lloret et al., 2007) and *S. terangae* (Martens et al., 2007). Bootstrap values (≥ 50%) obtained from NJ, ML and MP analyses are given on each related node.

Our other isolate OKN-10 appeared as sister to *M. loti* with 97.4% nucleotide sequence similarity and this relationship was supported with 76%, 75% and 51% bootstrap values in the NJ, ML and MP trees, respectively. Concordant with the phylogeny of 16S rDNA, *M. ciceri* and
M. cantuariense also appeared as related to the lineage above and this lineage was supported with bootstrap values higher than 50% in NJ and ML trees. OKN-3 revealed recA haplotype close to M. japonicum with 99.6% nucleotide sequence similarity and this relationship was supported with 100%, 100% and 95% bootstrap values in the NJ, ML, and MP trees, respectively. Additionally M. australicum, M. jarvisii, M. qingshengii and M. huakuii also appeared in the same lineage. On the other hand, M. erdmanii and M. opportunismum which were clearly related to this isolate in 16S rDNA phylogeny (Figure 1) did not appeared in the lineage in recA phylogeny.

DISCUSSION

Symbiotic diazotrophic bacteria commonly known as rhizobia is a popular subject for scientists all over the world because of their ecological and economical importance. So far some studies concerning rhizobia from Turkey have been published, however most of them are related to agronomic applications of rhizobial isolates (İçgen et al. 2002; Tüfenkci et al. 2006; Küçük & Kivanc, 2008a; Togay et al. 2008). On the other hand, only a couple of studies about the diversity of rhizobia in Turkey are available and the isolates in these studies only belong to genus Rhizobium (Küçük et al. 2006; Küçük & Kivanç, 2008b; Öğütçü et al. 2008; Ogutcu et al., 2009; Adiguzel et al., 2010; Gurkanli et al., 2013, 2014; Canik Orel et al. 2016). In this study, we characterized 5 rhizobial samples, OKN-1, -3, -4, -7, -10, isolated from root nodules of two wild legumes, Argyrolobium biebersteinii (Ball in Feddes) and Lotus angustissimus L., collected from Samsun province of Turkey. Phylogenetic analyses depending on 16S rDNA (Figure 1) clearly indicated the relationships of these isolates with the genus Mesorhizobium. From Turkey there are no records for any Mesorhizobium isolates identified using valid molecular methods, thus these isolates are the first ones. On the other hand, 16S rDNA did not provide sufficient information to associate our isolates with one of the valid Mesorhizobium species (Figure 1). Cordndant with our result, recent studies have clearly indicated that phylogenetic analysis solely depending on 16S rDNA do not fully resolve the evolutionary relationships among rhizobial isolates in species level due to the high degree of conservation of the gene (Mousavi et al., 2015). That’s why, we used a second housekeeping gene, recA, to identify our isolates. As a result, although isolates OKN-1 and OKN-4 from A. biebersteinii showed the same 16S rDNA haplotype with M. tarimense, M. gobiense and M. metallidurans (Figure 1), recA phylogeny clearly revealed their relationship with M. tarimense (Figure 2) which was originally identified from root nodules of Lotus sp. in Xinjiang, China (Han et al., 2008). To our knowledge, OKN-1 and OKN-4 are the only M. tarimense isolates reported after its description from China. Similarly, isolate OKN-3 from L. angustissimus L. showed the same 16S rDNA haplotype with M. erdmanii and M. japonicum (Figure 1), on the other hand in recA phylogeny, this isolate appeared as closely related to the later species (Figure 2). M. japonicum was identified with reclassification of two Lotus sp. nodulating bacteria from Japan and New Zealand which were formerly identified as M. loti (Martinez-Hidalgo et al., 2016). Since then, this species have not been reported from else where, thus this is the first report of M. japonicum from Turkey and Europe. Although, isolate OKN-10 from A. biebersteinii placed in the same lineage with M. loti, M. cicer and M. cantuariense in 16S rDNA (Figure 1) and recA trees (Figure 2), it did not showed enough similarity with none of these species to designate it to one of them, thus it is possible that this isolate is a new species. Likewise, OKN-7 probably represents a new Mesorhizobium species, since we could not designate this isolate to any of the available Mesorhizobium species according to 16S rDNA and recA phylogenies. These presumptions needs further investigations.

In conclusion, this study presents the first Mesorhizobium isolates identified using valid molecular methods from Turkey. Additionally, first reports of M. tarimense and M. japonicum from Turkey and Europe are also given in the study. These isolates are the first M. tarimense and M. japonicum isolates reported after the description of these two species from their original hosts and locations. This study also presents molecular evidences for two new Mesorhizobium species, but these presumptions needs further investigations.

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REFERENCES

Akaike, H. (1974). A new look at statistical model identification. IEEE Transactions on Automatic Control, 19, 716-723.

Adiguzel, A., Ogutcu, H., Baris, O., Karadayi, M., Gulluce, M.U. & Sahin, F. (2010). Isolation and characterization of Rhizobium strains from wild vetch collected from high altitudes in Erzurum-Turkey. Romanian Biotechnological Letters, 15, 5017-5024.

Alexandre, A., Laranjo, M., Young, J.P. & Oliveira, S. (2008). dnaJ is a useful phylogenetic marker for alpha proteobacteria. International Journal of Systematic and Evolutionary Microbiology, 58, 2839-2849.

Burdass, D. (2002). Rhizobium, root nodules and nitrogen fixation.

http://biblio.teluq.ca/LinkClick.aspx?fileticket=Zt58
Brewin, N.J., Beringer, J.E. & Johnston, A.W.B. (1980). Plasmid mediated transfer of host-range specificity between two strains of Rhizobium leguminosarum. Journal of General Microbiology, 120, 413-420.

Canik Orel, D., Karagöz, A., Durmaz, R. & Ertunç, F. (2016). Phenotypic and molecular characterization of Rhizobium vitis strains from vineyards in Turkey. Phytopathologia Mediterranea, 55, 41-53.

De Lajudie, P., Willems, A., Pot, B., Dewettinck, D., Maestrojuan, G., Neyra, M., Collins, M.D., Dreyfus, B., Kersters, K. & Gillis, M. (1994). Polyphasic taxonomy of Rhizobia: emendation of the genus Sinorhizobium and description of S. meliloti comb nov., S. saheli sp. nov., S. iringa sp. nov. International Journal of Systematic Bacteriology, 44, 715-733.

De Meyer, S.E., Tan, H.W., Heenan, P.B., Andrews, M. & Willems, A. (2015). Mesorhizobium waimense sp. nov. isolated from Sophora longicarinata root nodules and Mesorhizobium cantuariense sp. nov. isolated from Sophora microphylla root nodules. International Journal of Systematic and Evolutionary Microbiology, 65, 3419-3426.

Ditta, G., Virt, E., Palomares, A. & Kim, C. (1987). The nifA gene of Rhizobium meliloti is oxygen regulated. Journal of Bacteriology, 169, 3217-3223.

Gaunt, M.W., Turner, S.L., Rigottier-Gois, L., Lloyd-Macgilp, S.A. & Young, J.P.W., (2001). Phylogenies of atpD and recA support the small subunit rRNA-based classification of rhizobia. International Journal of Systematic and Evolutionary Microbiology, 51, 2037-2048.

Graham, P.H., Sadowsky, M.J., Keyser, H.H., Barnet, Y.M., Bradley, R.S., Cooper, J.E., De Ley, D.J., Jarvis, B.D.W., Roslyc, E.B., Strijdom, B.W. & Young, J.P.W. (1991). Proposed minimal standards for the description of new genera and species of root- and stem-nodulating bacteria. International Journal of Systematic Bacteriology, 41, 582-587.

Guan, S.H., Chen, W.F., Wang, E.T., Lu, Y.L., Yan, X.R., Zhang, X.X. & Chen, W.X. (2008). Mesorhizobium caraganae sp. nov., a novel rhizobial species nodulated with Caragana spp. in China. International Journal of Systematic and Evolutionary Microbiology, 58, 2646-2653.

Guindon, S. & Gascuel, O. (2003). A simple, fast and accurate algorithm to estimate large phylogenies by maximum-likelihood. Systematic Biology, 52, 696-704.

Gürkanli, C.T., Özkoç, İ. & Gündüz, İ. (2013). Genetic diversity of rhizobia nodulating common bean (Phaseolus vulgaris L.) in the Central Black Sea region of Turkey. Annals of Microbiology, 63, 971-987.

Gürkanli, C.T., Özkoç, İ. & Gündüz, İ. (2014). Genetic diversity of Vicia faba L. and Psism sativum L. nodulating rhizobia in the central Black Sea region of Turkey. Annals of Microbiology, 64, 99-112.

Han, T.X., Han, L.L., Wu, L.J., Chen, W.F., Sui, X.H., Gu, J.G., Wang, E.T. & Chen, W.X. (2008). Mesorhizobium gobiense sp. nov. and Mesorhizobium tarimense sp. nov., isolated from wild legumes growing in desert soils of Xinjiang, China. International Journal of Systematic and Evolutionary Microbiology, 58, 2610-2618.

Hall, T.A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Research, 41, 95-98.

İçgen, B., Özengiz, G. & Gürdal Alaeddinoglu, N. (2002). Evaluation of symbiotic effectiveness of various Rhizobium cicer strains. Research in Microbiology, 153, 369-372.

Jarvis, B.D.W., Van Berkum, P., Chen, W.X., Nour, S.M., Fernandez, M.P., Cleyet-Marel, J.C. & Gillis, M. (1997). Transfer of Rhizobium loti, Rhizobium huakuii, Rhizobium ciceri, Rhizobium mediterraneum, and Rhizobium tianshanense to Mesorhizobium gen. nov. International Journal of Systematic Bacteriology, 47, 895-898.

Küçük, Ç. & Kivanç, M. (2008a). The effect of Rhizobium spp. inoculation on seed quality of Bean in Turkey. Pakistan Journal of Biological Sciences, 11, 1856-1859.

Küçük, Ç. & Kivanç, M. (2008b). Preliminary characterization of Rhizobium strains isolated from chickpea nodules. African Journal of Biotechnology, 7, 772-775.

Lloret, L., Ormeno-Orrillo, E., Rincon, R., Martinez-Romero, J., Rogel-Hernandez, M.A. & Martinez-Romero, E. (2007). Ensifer mexicanus sp. nov. a new species nodulating Acacia angustissima (Mill.) Kuntze in Mexico. Systematic and Applied Microbiology, 30, 280-290.

Lodwig, E.M., Hosie, A.H.F., Bourdes, A., Findlay, K., Allaway, D., Karunakaran, R., Downie, J.A. & Poole, P.S. (2003). Amino-acid cycling drives nitrogen fixation in the legume-Rhizobium symbiosis. Nature, 422, 722-726.

Lu, Y.L., Chen, W.F., Wang, E.T., Han, L.L., Zhang, X.X., Chen, W.X. & Han, S.Z. (2009). Mesorhizobium shangrilense sp. nov., isolated from root nodules of Caragana species. International...
Journal of Systematic and Evolutionary Microbiology, 59, 3012-3018.

Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982). Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold spring Harbor, N.Y.

Martens, M., Delaere, M., Coopman, R., De, Vos P., Gillis, M. & Willems, A. (2007). Multilocus sequence analysis of Ensifer and related taxa. International Journal of Systematic and Evolutionary Microbiology, 57, 489-503.

Martínez-Hidalgo, P., Ramírez-Bahena, M.H., Flores-Felix, J.D., Rivas, R., Igual, J.M., Mateos, P.F., Martínez-Molina, E., Leon-Barrios, M., Peix, A. & Velazquez, E. (2015). Revision of the taxonomic status of type strains of *Mesorhizobium loti* and reclassification of strain USDA 3471T as the type strain of *Mesorhizobium erdmanii* sp. nov. and ATCC 33669T as the type strain of *Mesorhizobium jarvisii* sp. nov. International Journal of Systematic and Evolutionary Microbiology, 65, 1703-1708.

Martínez-Hidalgo, P., Ramírez-Bahena, M.H., Flores-Felix, J.D., Igual, J.M., Sanjuan, J., León-Barrios, M., Peix, A. & Velazquez, E. (2016). Reclassification of strains MAFF 303099T and R7A into *Mesorhizobium japonicu*n sp. nov. International Journal of Systematic and Evolutionary Microbiology, 66, 4936-4941.

Moreira, F.M.S., Haukka, K. & Young, J.P.W. (1998). Biodiversity of rhizobia isolated from a wide range of forest legumes in Brazil. Molecular Ecology, 7, 889-895.

Mousavi, S.A., Willems, A., Nesme, X., de Lajudie, P. & Lindström, K. (2015). Revised phylogeny of Rhizobiaceae: Proposal of the delineation of *Pararhizobium* gen. nov., and 13 new species combinations. Systematic and Applied Microbiology, 38, 84-90.

Nandasena, K.G., O’hara, G.W., Tiwari, R.P. & Howieson, J.G. (2006). Rapid in situ evolution of nodulating strains for *Biserrula pelecinus* L. through lateral transfer of a symbiosis island from the original mesorhizobial inoculant. Applied and Environmental Microbiology, 72, 7365-7367.

Nandasena, K.G., O’Hara, G.W., Tiwari, R.P., Willems, A. & Howieson, J.G. (2009). *Mesorhizobium australicum* sp. nov. and *Mesorhizobium contaminatum* sp. nov., isolated from *Biserrula pelecinus* L. in Australia. International Journal of Systematic and Evolutionary Microbiology, 59, 2140-2147

Nour, S.M., Fernandez, M.P., Normand, P. & Cleyet-Marel, J.C. (1994). *Rhizobium ciceri* sp. nov., consisting of strains that nodulate chickpeas (*Cicer arietinum* L.). International Journal of Systematic Bacteriology, 44, 511-522.

Ogutcu, H., Adguzel, A., Gulluce, M., Karadayi, M. & Sahin, F. (2009). Molecular characterization of *Rhizobium* strains isolated from wild chickpeas collected from high altitudes in Erzurum-Turkey. Romanian Biotechnological Letters, 14, 4294-4300.

Ögüçü, H., Algür, Ö.F., Elkoça, E. & Kantar, F. (2008). The Determination of Symbiotic Effectiveness of Rhizobium Strains Isolated from Wild Chickpeas Collected from High Altitudes in Erzurum. Turkish Journal of Agriculture and Forestry, 32, 241-248.

Oyaizu, H., Matsumoto, S., Minamisawa, K. & Gamou, T. (1993). Distribution of Rhizobia in leguminous plants surveyed by phylogenetic identification. Journal of General and Applied Microbiology, 39, 339-354.

Posada, D. (2008). jModel: phylogenetic model averaging. Molecular Biology and Evolution, 25, 1253-1256.

Raymond, J., Siefert, J.L., Staples, C.R. & Blankenship, R.E. (2004). The Natural history of nitrogen fixation. Molecular Biology and Evolution, 21, 541-554.

Sawada, H., Kuykendall, L.D. & Young, J.M. (2003). Changing concepts in the systematics of bacterial nitrogen-fixing legume symbionts. Journal of General and Applied Microbiology, 49, 155-179.

Shamseldin, A., Abdelkhalak, A. & Sadowsky, M.J. (2017). Recent changes to the classification of symbiotic, nitrogen-fixing, legume-associating bacteria: a review. *Symbiosis*, 71, 91-109.

Somasegaran, P. & Hohen, H.J. (1985). Methods in legume-Rhizobium technology. United States Agency for International Development, USA.

Sprent, J.L. (2007). Evolving ideas of legume evolution and diversity: a taxonomic perspective on the occurrence of nodulation. New Phytologist, 174, 11-25.

Swafford, D.L. (1998). PAUP* Phylogenetic analysis using parsimony (*and other methods). Version 4 beta 10. Sinauer Associates, Sunderland, Massachusetts.

Temizkan, G. & Arda, N. (2004). Moleküler biyolojide kullanılan yöntemler. Nobel Tıp Kitabevleri, İstanbul.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997). The ClustalX-Windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research, 25, 4876-4882.

Togay, N., Togay, Y., Cimrin, K.M. & Turan, M. (2008). Effects of *rhizobium* inoculation, sulfur and phosphorus applications on yield, yield components and nutrient uptakes in chickpea (*Cicer arietinum* L.). African Journal of Biotechnology, 7, 776-782.

Tufenkeci, S., Erman, M. & Sonmez, F. (2006). Effects of phosphorus and nitrogen applications and *Rhizobium* inoculation on the yield and nutrient uptake of...
sainfoin (Onobrychis viciifolia L.) under irrigated conditions in Turkey. New Zealand Journal of Agricultural Research, 49, 101-105.

Vidal, C., Chantreuil, C., Berge, O., Maure, L., Escarre, J., Bena, G., Brunel, B. & Cleyet-Marel, J.C. (2009). Mesorhizobium metallidurans sp. nov., a metal-resistant symbiont of Anthyllis vulneraria growing on metallicolous soil in Languedoc, France. International Journal of Systematic and Evolutionary Microbiology, 59, 850-855.

Vincent, J.M. (1970). A manual for the Practical Study of the Root-Nodule Bacteria. Blackwell Scientific Publications, Oxford and Edinburgh.

Vinuesa, P., Silva, C., Lorite, M.J., Izaguirre-Mayora, M.L., Bedmar, E.J. & Martinez-Romero, E. (2005). Molecular systematics of rhizobia based on maximum likelihood and Bayesian phylogenies inferred from rrs, atpD, recA and nifH sequences, and their use in the classification of Sesbania microsymbionts from Venezuelan wetlands. Systematic and Applied Microbiology, 28, 702-716.

Wang, E.T., van Berkum, P., Sui, X.H., Beyene, D., Chen, W.X. & Martinez-Romero, E. (1999). Diversity of rhizobia associated with Amorpha fruticosa isolated from Chinese soils and description of Mesorhizobium amorphae sp. nov. International Journal of Systematic and Evolutionary Microbiology, 49, 51-65.

Willems, A. & Collins, M.D. (1993). Phylogenetic analysis of rhizobia and agrobacteria based on 16S rRNA gene sequences. International Journal of Systematic Bacteriology, 43, 305-313.

Willems, A. (2006). The taxonomy of rhizobia: an overview. Plant and Soil, 287, 3-14.

Weisburg, W.G., Barns, S.M., Pelletier, D.A. & Lane, D.J. (1991). 16S Ribosomal DNA amplification for phylogenetic study. Journal of Bacteriology, 173, 697-703.

Zhang, X., Harper, R., Karsisto, M. & Lindstrom, K. (1991). Diversity of Rhizobium bacteria isolated from the root nodules of leguminous trees. International Journal of Systematic Bacteriology, 41, 104-113.

Zhang, X.X., Guo, X.W., Terefework, Z., Paulin, L., Cao, Y.Z., Hu, F.R., Lindstrom, K. & Li, F.D. (1999). Genetic diversity among rhizobial isolates from field-grown Astragalus sinicus of Southern China. Systematic and Applied Microbiology, 22, 312-320.

Zhang, J.J., Liu, T.Y., Chen, W.F., Wang, E.T., Sui, X.H., Zhang, X.X., Li, Y., Li, Y. & Chen, W.X. (2012). Mesorhizobium muleiense sp. nov., nodulating with Cicer arietinum L. International Journal of Systematic and Evolutionary Microbiology, 62, 2737-2742.

Zheng, W.T., Li, Y. Jr., Wang, R., Sui, X.H., Zhang, X.X., Zhang, J.J., Wang, E.T. & Chen, W.X. (2013). Mesorhizobium qingshengii sp. nov., isolated from effective nodules of Astragalus sinicus. International Journal of Systematic and Evolutionary Microbiology, 63, 2002-2007.

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