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Phylogenomic Review of Root Nitrogen-Fixing Symbiont Population Nodulating Northwestern African Wild Legumes

Mokhtar Rejili, Mohamed Ali Ben Abderrahim and Mohamed Mars

Abstract

The present review discusses the phylogenomic diversity of root nitrogen-fixing bacteria associated to wild legumes under North African soils. The genus *Ensifer* is a dominant rhizobium lineage nodulating the majority of the wild legumes, followed by the genus *Rhizobium* and *Mesorhizobium*. In addition, to the known rhizobial genera, two new *Microvirga* and *Phyllobacterium* genera were described as real nodulating and nitrogen-fixing microsymbiotes from *Lupinus* spp. The promising rhizobia related to nitrogen fixation efficiency in association with some legumes are shared. Phylogenetic studies are contributing greatly to our knowledge of relationships on both sides of the plant-bacteria nodulation symbiosis. Multiple origins of nodulation (perhaps even within the legume family) appear likely. However, all nodulating flowering plants are more closely related than previously suspected, suggesting that the predisposition to nodulate might have arisen only once. The origins of nodulation, and the extent to which developmental programs are conserved in nodules, remain unclear, but an improved understanding of the relationships between nodulin genes is providing some clues.

Keywords: rhizobia, North Africa, symbiosis, legumes, phylogenomic

1. Introduction

Africa has a vast array of indigenous legumes, ranging from large rain forest trees to small annual herbs [1]. However, in recent years, there has been a tendency in agriculture and forestry to use exotic species for crops and wood. As has been pointed out several times over nearly 30 years, most recently [2], by the US National Academy of Sciences, this ignores the potential of the native species, which are arguably better adapted to their environment. For this review, the nodulated indigenous legume genera in Northwestern Africa with known uses have been selected to illustrate the problems and potential for their better exploitation.

The wild legume flora in Northwestern Africa is rich, with great specific and infraspecific diversity [3]. The overgrazing and expansion of agriculture has gradually

led to the regression and extinction of many pastoral and forage species. In addition, desertification causes disturbance of plant-microbe symbioses, which are a critical ecological factor in helping further plant growth in degraded ecosystems [4]. In this context, the establishment of indigenous pastoral legume species associated with their appropriate symbiotic bacterial partners may be of increased value for success in soil fertility restoration. Biological N₂ fixation (BNF) is the major way for N input into desert ecosystems. Rhizobium-legume symbioses represent the major mechanism of BNF in arid lands, compared with the N₂-fixing heterotrophs and associative bacteria [5, 6] and actinorhizal plants [7, 8]. Deficiency in mineral N often limits plant growth, and so symbiotic relationships have evolved between plants and a variety of N₂-fixing organisms [9]. The symbiotically fixed N₂ by the association between rhizobium species and the legumes represents a renewable source of N for agriculture. Values estimated for various legume crops and pasture species are often impressive [10]. In addition to crop legumes, the nodulated wild (herb and tree) legumes have potential for nitrogen fixation and reforestation and to control soil erosion [11]. It has been reported that a novel, suitable wild legume-rhizobia associations are useful in providing a vegetational cover in degraded lands [12].

Considering the major ecological importance of many wild legumes such as *Retama* sp., *Acacia* sp., *Lotus* sp., *Lupinus* sp., *Medicago* sp., etc. in Northwest Africa by their important role in soil fertility maintenance, coverage, and dune stabilization, the present chapter proposes to review the phylogenomic diversity of root nitrogen-fixing symbiont population nodulating Northwestern African wild legumes listed in the bibliography, some of which are common and play important ecological and pastoral roles, but others are rare and endangered. As well as the host legumes, the nodule endosymbionts also vary widely in Africa and include newly described members of both α and β branches of the Proteobacteria, now often referred to as α - or β -rhizobia, even though they do not have “rhizobium” as part of their generic names [13].

Therefore, understanding the nature of indigenous populations of rhizobia-nodulating wild legumes is of considerable agricultural significance. It is also of interest to identify a wider variety of bacterial strains in a bid to define new strains for the production of inoculants for smallholder farms.

2. Genetics and functional genomics of legume nodulation

The interaction between rhizobia and legumes in root nodules is an essential element in sustainable agriculture, as this symbiotic association is able to enhance biological fixation of atmospheric nitrogen (N₂) and is also a paradigm in plant-microbe signaling [14–16]. The knowledge of the whole genome would allow the specific features of each rhizobium to be identified. The prominent feature of this group of bacteria is their molecular dialog with plant hosts, an interaction that is enabled by the presence of a series of symbiotic genes encoding for the synthesis and export of signals triggering organogenetic and physiological responses in the plant [17, 18]. In recent years, significant progress has been made in resolving the complex exchange of signals responsible for nodulation through genome assembly, mutational and expression analysis, and proteome characterization of legumes [14, 19, 20] and rhizobia [15, 21–23].

3. Phylogenomic of wild legume root nitrogen-fixing symbionts

The known diversity of rhizobia increases annually and is the subject of several reviews, the most recent and comprehensive being that of [24]. It is not our intention to revisit this subject nor the genetic basis of nodulation [25, 26], the horizontal

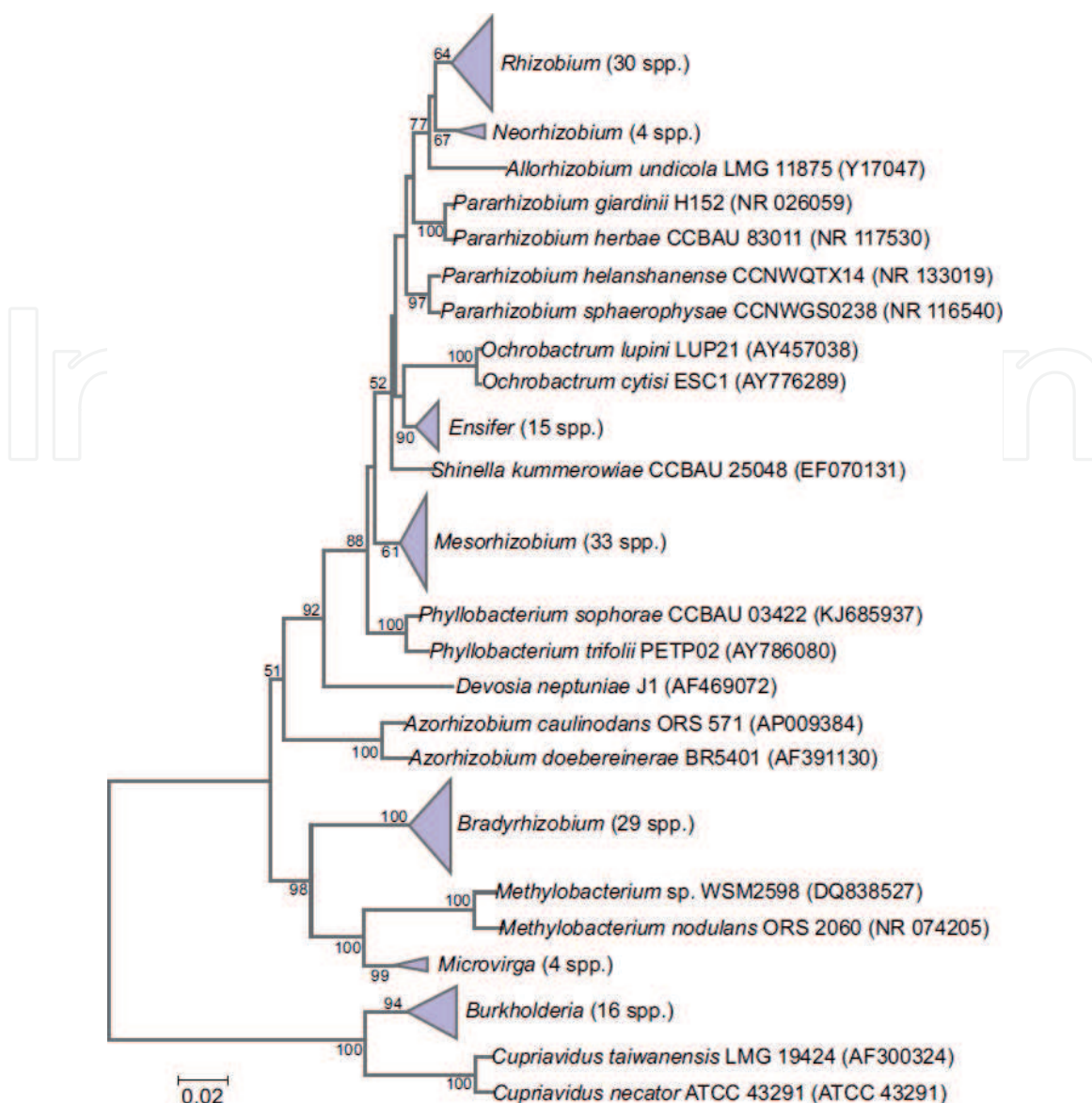


Figure 1.
Phylogenetic tree showing the relationships of currently described genera and species of alpha- and beta-rhizobia, based on aligned sequences of the 16S rRNA gene (1341-bp internal region) (adapted from [28]).

transfer of symbiosis-related genes [23], or the symbiovar concept [27] but instead to attempt to link, where possible, rhizobial genotypes with their geographical locations and/or legume tribes/genera. At the time of writing, rhizobia consist of a diverse range of genera in the Alphaproteobacterial and Betaproteobacterial classes and are termed “alpha-rhizobia” and “beta-rhizobia,” respectively (**Figure 1**).

3.1 The genus *Bradyrhizobium* (*Bradyrhizobiaceae*)

The *Bradyrhizobium* genus was described by Jordan in 1982 [29]. It currently consists of nine rhizobia species.

For the *Loteae* tribe, previous studies found that *Lotus palustris* and *L. purpureus* species from Algeria were nodulated by *Bradyrhizobium lupini*, and *L. pedunculatus* by *B. japonicum* [30]. However, *L. creticus* ssp. *maritimus* is nodulated by both [30]. At Tunisia, *L. roudairei* microsymbiont is closely related to *B. japonicum* [31]. For the *Acacieae* tribe, two studies reported that rhizobial strains associated to the *Acacia saligna*, an Australian introduced species, belonged to the genus *Bradyrhizobium* genus under Algerian and Moroccan soils [32–34]. For the *Genisteeae* tribe, it has been noticed that *Bradyrhizobium* is the dominant genus of

symbiotic nitrogen-fixing bacteria associated with *Retama* species in North Africa: *Retama monosperma*, *R. raetam*, and *R. sphaerocarpa* (Algeria: [35, 36]; Morocco: [37]). Recently, the novel *B. retamae* species, in which groups with *B. elkanii* and *B. pachyrhizi* and related *B. lablabi* and *B. jicamae* type strains are included in *Bradyrhizobium* group II [38], has been isolated from *R. sphaerocarpa* and *R. monosperma* in Morocco [37]. For the genus *Cytisus*, two studies reported that *Cytisus villosus* is nodulated by *B. cytisi* sp. nov. and *B. rifense* sp. nov. in Morocco [39, 40] and by genetically diverse *Bradyrhizobium* strains in Algeria belonging to *B. japonicum* and *B. canariense* and to new lineage within the *Bradyrhizobium* genus [41]. Fifty-two strains isolated from root nodules of the Moroccan shrubby legume *Cytisus triflorus* were genetically characterized, and results showed that it is nodulated by *Bradyrhizobium* strains, with 99% homology with *Bradyrhizobium* genosp. AD [42]. For the genus *Lupinus*, some endosymbiotic bacteria of *L. luteus* and *L. micranthus* from Tunisia and Algeria belonged to *B. lupini*, *B. canariense*, *B. valentinum*, *B. cytisi/B. rifense*, *B. japonicum*, *B. elkanii*, and *B. retamae* [43–45].

3.2 The genus *Mesorhizobium* (*Phyllobacteriaceae*)

The genus *Mesorhizobium* was described by Jarvis et al. [46]. Several *Rhizobium* species were transferred to this genus. It currently consists of 21 rhizobia species.

For subtribe *Astragalinae* (Coluteinae Clade), Guerrouj et al. [37] reported that rhizobial symbiont of *Astragalus gombiformis* in Eastern Morocco is closely related to *M. camelthorni*. A polyphasic approach analysis indicated that bacterial strains isolated from the pasture legume *Biserrula pelecinus* growing in Morocco belong to the genus *Mesorhizobium*. At Tunisia, Mahdhi et al. [47] showed that five strains isolated from *Astragalus corrugatus* were phylogenetically related to *M. temperatum* and to *Mesorhizobium* sp. From the tribe *Galegeae* (subtribe *Coluteinae*), Ourarhi et al. [48] reported that *Colutea arborescens* is nodulated by diverse rhizobia in Eastern Morocco, among them, the genus *Mesorhizobium*. For the *Loteae* tribe, *M. alhagi* as well as *M. temperatum* were isolated, at Tunisia, from *Lotus creticus* [49–51]. Zakhia et al. [31] reported that *Lotus argenteus* microsymbiotes are closely related to *M. mediterraneum* in the infra-arid zone of Tunisia. Roba et al. [52] reported that *M. delmotii* and *M. prunaredense* are two new rhizobial species nodulating *Anthyllis vulneraria* growing on Tunisian soils. From the *Acacieae* tribe, Boukhatem et al. [33] reported that rhizobial strains associated to the *Acacia saligna*, an Australian introduced species, to *A. ehrenbergiana* and *F. albida* belonged to *M. mediterraneum* under Algerian soils. From the *Genisteae* tribe, the genetic diversity of *Genista saharae* microsymbionts in the Algerian Sahara reported that they belonged to *M. camelthorni* [53]. For the *Mimoseae* tribe, root-nodulating bacteria associated to *Prosopis farcta* growing in the arid regions of Tunisia were assigned to the genus *Mesorhizobium* [54]. From the *Hedysareae* tribe, Zakhia et al. [31] reported that one strain isolated from *Ebenus pinnata* root nodules is closely related to *M. ciceri* in the infra-arid zone of Tunisia.

3.3 The genus *Rhizobium* (*Rhizobiaceae*)

The genus *Rhizobium* was the first named (from Latin meaning “root living”), and for many years this was a “catch all” genus for all rhizobia. Some species were later moved in to new genera based on phylogenetic analyses [55]. It currently consists of 49 rhizobial species.

For *Galegae* tribe, Zakhia et al. [31] reported that rhizobial symbionts of *Astragalus gombiformis*, *A. armatus*, and *A. cruciatus* are closely related to *Rhizobium mongolense*, *R. leguminosarum*, and *R. galegae*, in the infra-arid zone of Tunisia. From

Genisteae tribe, it was shown that strains from Tunisia nodulating *Argyrolobium uniflorum* are closely affiliated to *R. giardinii*, *Calicotome villosa* to *R. mongolense*, and *Genista microcephala* to *R. mongolense* and *R. leguminosarum* [31]. Mahdi et al. [56–58] reported that strains nodulating *Genista saharae* and *Retama retam* are members of the genus *Rhizobium*. Nonetheless, there are reports indicating that members of the genus *Rhizobium* nodulate *Adenocarpus decorticans* and *Cytisus arboreus* at Morocco [59]. For the *Loteae* tribe, *R. leguminosarum* and *R. mongolense* were isolated, at Tunisia, from *Anthyllis henoniana*, *R. leguminosarum* from *Coronilla scorpioides*, and *R. mongolense* from *Lotus creticus* [31]. Rejili et al. [51] reported that *Lotus creticus* microsymbiotes are closely related to *R. huautlense* in the arid areas of Tunisia. Bacterial strains isolated from root nodules of *Scorpiurus muricatus* sampled from different regions of western Algeria are affiliated to *R. vignae*, *R. radiobacter*, and *R. leguminosarum* [60]. For the *Trifolieae* tribe, *R. galegae* species was isolated, in Tunisia and Algeria, from *Medicago marima* and *M. truncatula* [31]. In Algeria, Merabet et al. [61] reported that *Medicago ciliaris* and *M. polymorpha* are nodulated by *Rhizobium* sp. Similarly, genetic diversity of rhizobia from annual *Medicago orbicularis* showed that they are affiliated to *Rhizobium tropici* [62]. For the *Vicieae* tribe, *R. leguminosarum* species was isolated from *Lathyrus numidicus* [31]. Mahdhi et al. [63] reported that *Vicia sativa* isolates from Tunisia had 16S rDNA type identical to that of the reference *R. leguminosarum*. From *Acacieae*, Boukhatem et al. [33] reported that bacteria-nodulating *Acacia saligna* and *A. seyal* under Algerian soils are affiliated to the *R. tropici* clade and *R. sullae* clade, respectively. On the other hand, the same study mentioned that five bacterial isolates, all from *A. saligna*, formed a separate clade in the vicinity of the *R. galegae*-*R. huautlense*-*R. loessense* branch [33]. The same authors showed that the *R. leguminosarum* reference strain was represented by five *A. karroo* isolates and five *A. seyal* isolates [33]. At Tunisia, the genetic diversity of root nodule bacteria associated to *Hedysarum coronarium* (*sulla*), from *Hedysareae* tribe, showed that they are closely related to *R. sullae* [64]. Similarly, Ezzakkioui et al. [65] indicated that the strains from the Moroccan *Hedysarum flexuosum* legume had 99.75–100% identity with *R. sullae*.

3.4 The genus *Ensifer* (*Sinorhizobium*) (*Rhizobiaceae*)

The genera *Sinorhizobium* and *Ensifer* were recently recognized as forming a single phylogenetic clade [66, 67] and are now united, and all species of the genus *Sinorhizobium* have been transferred to the genus *Ensifer*, in line with rule 38 of the Bacteriological Code [68, 69]. The genus currently consists of 17 species.

Bacteria belonging to *Ensifer* genus are widely distributed in arid regions of Tunisia. From the *Loteae* tribe, *E. meliloti* and *E. numidicus* were isolated, at Tunisia, from *Lotus creticus* [49–51, 69] and *Rhizobium* sp. from *Hippocrepis areolata* [47]. From the *Acacieae* tribe, genetic characterization of rhizobial bacteria-nodulating *Acacia tortilis* subsp. *raddiana*, *A. gummifera*, *A. cyanophylla*, *A. karroo*, *A. ehrenbergiana*, and *A. horrida* in Tunisia, Algeria, and Morocco reported that they belonged to the species *E. meliloti*, *E. garamanticus*, and *E. numidicus* and *Ensifer* sp. [31, 33, 70–72, 73]. At Algeria, isolates from four different host species, namely, *A. karroo*, *A. ehrenbergiana*, *A. saligna*, and *A. tortilis*, were closely related to *E. fredii*, *E. teranga*, and *E. kostiense* reference strains [33]. For the *Mimoseae* tribe, 40 isolates associated to *Prosopis farcta* growing in the arid regions of Tunisia belonged to *E. meliloti*, *E. xinjiangense*/*E. fredii*, and *E. numidicus* species [54]. For the *Trifolieae* tribe, strains nodulating different *Medicago* species in Tunisia, Algeria, and Morocco such as *M. sativa*, *M. arborea*, *M. truncatula*, *M. ciliaris*, *M. laciniata*, *M. polymorpha*, *Medicago arabica*, *M. marima*, *Medicago littoralis*, and *M. scutella* are associated to *E. meliloti*, *E. medicae*, or *E. garamanticus* [31, 61, 62, 69, 74–79]. Similarly, *Ononis natrix*

Subfamily tribe	Genus	Species	Symbiont	Geographic origin
Mimosoideae				
Acacieae	Acacia	A. cyanophylla	E. meliloti, E. fredii, Ensifer sp.	Tunisia, Morocco
		A. gummifera	E. meliloti, E. garamanticus, E. numidicus, Ensifer sp.	Tunisia, Morocco
		A. horrida	E. meliloti, E. garamanticus, E. numidicus, Ensifer sp.	Tunisia, Morocco
		A. tortilis raddiana	E. meliloti, E. garamanticus, E. numidicus, Ensifer sp.	Tunisia, Morocco
		A. saligna	Bradyrhizobium sp., Mesorhizobium sp., Rhizobium sp., Ensifer sp.	Algeria, Morocco
		A. ehrenbergiana	Mesorhizobium sp., Ensifer sp.	Algeria
		A. karroo	Rhizobium sp., Ensifer sp.	Algeria
		A. nilotica	Rhizobium sp.	Algeria
		A. seyal	Rhizobium sp.	Algeria
		F. albida	Mesorhizobium sp.	Algeria
Mimosae	Prosopis	P. farcta	Mesorhizobium sp., E. meliloti, E. xinjiangense, E. fredii, E. numidicus	Tunisia
Papilionoideae				
Galegae	Astragalus	A. armatus	R. mongolense, R. leguminosarum, R. galegae	Tunisia
		A. cruciatus	R. mongolense, R. leguminosarum, R. galegae	Tunisia
		A. corrugatus	M. temperatum, Mesorhizobium	Tunisia
		A. gombiformis	M. camelthorni, R. mongolense, R. leguminosarum, R. galegae	Morocco, Tunisia
	Biserrula	B. pelecinus	Mesorhizobium	Morocco
	Colutea	C. arborescens	Mesorhizobium	Morocco
Genisteae	Argyrolobium	A. uniflorum	R. giardinii	Tunisia
	Adenocarpus	A. decorticans	Rhizobium	Morocco
	Calicotome	C. villosa	R. mongolense	Morocco
	Cytisus	C. arboreus	Bradyrhizobium sp.	Morocco
		C. triflorus	Bradyrhizobium	Morocco
		C. villosus	B. cytisi, B. rifense, B. japonicum, B. canariense	Algeria, Morocco
		Lupinus	L. luteus	B. lupini, B. canariense, B. valentinum, B. cytisi, B. rifense, B. japonicum, B. elkanii, B. retamae, Microvirga

Subfamily tribe	Genus	Species	Symbiont	Geographic origin
		<i>L. micranthus</i>	<i>B. lupini</i> , <i>B. canariense</i> , <i>B. valentinum</i> , <i>B. cytisi</i> , <i>B. rifense</i> , <i>B. japonicum</i> , <i>B. elkanii</i> , <i>B. retamae</i> , <i>Microvirga</i> , <i>Phyllobacterium</i>	Algeria, Tunisia
	<i>Genista</i>	<i>G. microcephala</i>	<i>R. mongolense</i> , <i>R. leguminosarum</i> , <i>Rhizobium</i>	Tunisia
		<i>G. saharae</i>	<i>M. camelthorni</i>	Algeria
	<i>Retama</i>	<i>R. monosperma</i>	<i>B. retamae</i>	Algeria, Morocco
		<i>R. raetam</i>	<i>B. retamae</i> , <i>Rhizobium</i>	Algeria, Tunisia, Morocco
		<i>R. sphaerocarpa</i>	<i>B. retamae</i>	Algeria, Morocco
Hedysareae	<i>Hedysarum</i>	<i>H. carnosum</i>	<i>E. meliloti</i>	Tunisia
		<i>H. flexuosum</i>	<i>R. sullae</i>	Morocco
		<i>H. coronarium</i>	<i>R. sullae</i>	Tunisia
		<i>H. spinosissimum</i>	<i>E. meliloti</i>	Tunisia
	<i>Ebenus</i>	<i>E. pinnata</i>	<i>M. ciceri</i>	Tunisia
Loteae	<i>Anthyllis</i>	<i>A. henoniana</i>	<i>R. leguminosarum</i> , <i>R. mongolense</i>	Tunisia
		<i>A. vulneraria</i>	<i>M. delmotii</i> , <i>M. prunaredense</i>	Tunisia
	<i>Coronilla</i>	<i>C. scorpioides</i>	<i>R. leguminosarum</i>	Tunisia
	<i>Hippocrepis</i>	<i>H. areolata</i>	<i>Rhizobium</i>	Tunisia
		<i>H. bicontorta</i>	<i>E. meliloti</i>	Tunisia
	<i>Lotus</i>	<i>L. argenteus</i>	<i>M. mediterraneum</i>	Tunisia
		<i>L. creticus</i>	<i>B. lupini</i> , <i>B. japonicum</i> , <i>M. alhagi</i> , <i>M. temperatum</i> , <i>R. mongolense</i> , <i>R. huautlense</i> , <i>E. meliloti</i> , <i>E. numidicus</i>	Algeria, Tunisia
		<i>L. palustris</i>	<i>B. lupini</i>	Algeria
		<i>L. pedunculatus</i>	<i>B. japonicum</i>	Algeria
		<i>L. purpureus</i>	<i>B. lupini</i>	Algeria
		<i>L. pusillus</i>	<i>M. alhagi</i> , <i>M. temperatum</i> , <i>E. meliloti</i>	Tunisia
		<i>L. roudairei</i>	<i>B. japonicum</i>	Tunisia
	<i>Scorpiurus</i>	<i>S. muricatus</i>	<i>R. vignae</i> , <i>R. radiobacter</i> , <i>R. leguminosarum</i>	Algeria
Trifolieae	<i>Medicago</i>	<i>M. arabica</i>	<i>E. meliloti</i> , <i>E. medicae</i> , and <i>E. garamanticus</i>	Morocco
		<i>M. arborea</i>	<i>E. meliloti</i> , <i>E. medicae</i> , and <i>E. garamanticus</i>	Morocco

Subfamily tribe	Genus	Species	Symbiont	Geographic origin
		<i>M. ciliaris</i>	<i>Rhizobium</i> , <i>E. meliloti</i> , <i>E. medicae</i> , and <i>E. garamanticus</i>	Algeria
		<i>M. marima</i>	<i>R. galegae</i>	Algeria, Tunisia
		<i>M. laciniata</i>	<i>E. meliloti</i> , <i>E. medicae</i> , and <i>E. garamanticus</i>	Tunisia
		<i>M. littoralis</i>	<i>E. meliloti</i> , <i>E. medicae</i> , and <i>E. garamanticus</i>	Tunisia
		<i>M. orbicularis</i>	<i>R. tropici</i>	Tunisia, Algeria, Morocco
		<i>M. polymorpha</i>	<i>Rhizobium</i> , <i>E. meliloti</i>	Tunisia, Algeria, Morocco
		<i>M. sativa</i>	<i>E. meliloti</i> , <i>E. medicae</i>	Tunisia, Algeria, Morocco
		<i>M. scutella</i>	<i>E. meliloti</i>	Algeria, Tunisia
		<i>M. truncatula</i>	<i>R. galegae</i> , <i>E. meliloti</i> , <i>E. medicae</i>	Tunisia, Algeria, Morocco
	<i>Melilotus</i>	<i>M. indicus</i>	<i>E. meliloti</i>	Algeria
	<i>Ononis</i>	<i>O. natrix</i> ssp. <i>filifolia</i>	<i>E. meliloti</i>	Tunisia
	<i>Trigonella</i>	<i>T. maritima</i>	<i>E. meliloti</i>	Tunisia
Vicieae	<i>Lathyrus</i>	<i>L. numidicus</i>	<i>R. leguminosarum</i>	Tunisia
	<i>Vicia</i>	<i>V. sativa</i>	<i>R. leguminosarum</i>	Tunisia

Table 1.
Recapitulative results of root nodule symbionts from Northwestern African wild legumes.

and *Trigonella maritima* are nodulated by *E. meliloti* [31, 63]. Nodule rhizobia of *Melilotus indicus* growing in the Algerian Sahara are affiliated to *E. meliloti* [78]. *E. meliloti* and *E. numidicus* strains were isolated from the Genisteae tribe such as *Argyrolobium uniflorum*, *Retama raetam*, and *Genista saharae* [56–58, 69]. For *Galegae* tribe, Mahdhi et al. [47] reported that rhizobial symbionts of *Astragalus corrugatus* are closely related to *E. meliloti* under Tunisian soils. From the *Hedysareae* tribe, Mahdhi et al. [35] reported that strains isolated from *Hedysarum spinosissimum* root nodules are closely related to *E. meliloti* in the infra-arid zone of Tunisia.

3.5 The genus *Neorhizobium* (*Rhizobiaceae*)

The genus *Neorhizobium* was proposed by Mousavi et al. [80] as an alternative to solve the issue of grouping the members of this genus with *Agrobacterium* and *Rhizobium* genera. The genetic diversity of the Algerian legume *Genista saharae* isolates was assessed, and results reported that they are affiliated to *Neorhizobium alkalisoli*, *N. galegae*, and *N. huautlense* [53]. Several studies reported that *N. galegae* is isolated from different legumes in Tunisia such as *Astragalus* sp. [31, 54], *Argyrolobium*

uniflorum [31], *Anthyllis henoniana* [31], *Lotus creticus* [31, 50], *Medicago marima*, and *M. truncatula* [31]. Rejili et al. [51] reported that *Lotus creticus* is also nodulated by *N. huautlense* in the arid areas of Tunisia. For *Galegae* tribe, Mahdhi et al. [47] reported that rhizobial symbionts of *Astragalus corrugatus* are closely related to *N. galegae* under Tunisian soils.

3.6 The genus *Phyllobacterium* (*Phyllobacteriaceae*)

The *Phyllobacterium* genus comprises of bacteria that are well-known for their epiphytic and endophytic associations with plants [81]. Nonetheless, root-nodulating and nitrogen-fixing *Phyllobacterium* was described in Tunisia, in the nodules of genistoid legume *Lupinus micranthus* [44, 45]. Prior to this finding, endophytic *Phyllobacterium* strains were identified on the nodules of the Tunisian legumes *Genista saharae*, *Lotus creticus*, and *L. pusillus* [51, 57], but they are lacking the ability to form nodules.

3.7 The genus *Microvirga* (*Methylobacteriaceae*)

The genus *Microvirga* which comprises soil and water saprophytes was included in the alphaproteobacterial lineage of root-nodule bacteria only in 2012, although the first symbiotic strains were detected in nodules of *Lupinus texensis* [82–84]. Recently, *Microvirga* strains were only isolated from *L. micranthus* and *L. luteus* in Tunisia, belonging to the *Genisteae* tribe [44, 45].

Table 1 shows the root nodule symbionts from Northwestern African wild legumes.

4. Promising nitrogen-fixing rhizobia

The root nodule symbiosis established between legumes and rhizobia is an exquisite biological interaction responsible for fixing a significant amount of nitrogen in terrestrial ecosystems. The success of this interaction depends on the recognition of the right partner by the plant within the richest microbial ecosystems on Earth, the soil. Recent metagenomic studies of the soil biome have revealed its complexity, which includes microorganisms that affect plant fitness and growth in a beneficial, harmful, or neutral manner. In this complex scenario, understanding the molecular mechanisms by which legumes recognize and discriminate rhizobia from pathogens, but also between distinct rhizobia species and strains that differ in their symbiotic performance, is a considerable challenge.

By symbiotic efficiency and properties, strains isolated from wild legumes varied in their symbiosis effectiveness with their host plant of origin. A great diversity among and within isolates was reported by many authors. This symbiotic diversity within and between isolates growing in diverse geographical areas was also defined by Tinick and Hadobas [85] for other legume plants. All strains were capable of nodulation. Mahdhi et al. [55, 86] reported that two *Retama raetam* isolates RB3 and RM4 (*Rhizobium*) gave the highest nodule numbers per plant, 26 (± 2.053) and 27 (± 0.997), respectively. The effective strain LAC765 (*Ensifer*) was isolated from *Lotus creticus* with a 91.46 ($\pm 0.01\%$) dry biomass of the T_N control [50]. The dry matter of the aerial part is considered a criterion for assessing the efficiency of a given strain; a highly significant correlation between these two parameters has been reported. Results related to symbiotic efficiency showed that among 45 tested isolates, 20 isolates are highly efficient (relative effectiveness $\geq 70\%$), 20 isolates are partially effective ($60\% \leq$ relative effectiveness $< 70\%$), and 5 isolates are inefficient (relative

effectiveness <60%). The strain GN29 isolated from *Genista saharae*, affiliated to *Rhizobium* genus, is considered inefficient (relative effectiveness = 32.29%). Among the 20 isolates considered highly efficient, 5 isolates were isolated from *Retama retam*, five from *Lotus* sp., 4 from *Genista saharae*, 3 from *Vicia sativa*, 2 from *Argyrolobium uniflorum* and 2 from *Trigonella maritima*. From the 20 highly efficient isolates, 13 isolates belong taxonomically to *Ensifer* sp., 6 to *Rhizobium* sp., and one to *Mesorhizobium* sp.

5. Conclusion

The Mediterranean basin is a hotspot place of legume diversity and the center of diversification of many of them. Our review contributes to enlarge our knowledge on the LNB-legume symbioses. We evidenced the biodiversity among bacteria-nodulating wild legumes in Northwestern Africa and unknown associations were found. Several groups may represent new genospecies to be further characterized to assess their taxonomical status. This work thus opens further interesting perspectives and makes new models available for evolutionary studies and for understanding mechanisms involved in nitrogen-fixing symbiosis.

Conflict of interest

The authors declare that they have no conflict of interest.

Author details


Mokhtar Rejili^{1*}, Mohamed Ali BenAbderrahim² and Mohamed Mars¹

¹ Laboratory of Biodiversity and Valorization of Arid Areas Bioresources (BVBA), Faculty of Sciences of Gabes, Erriadh, Zrig, Tunisia

² Arid and Oases Cropping Laboratory, Arid Area Institute, Gabes, Tunisia

*Address all correspondence to: rejili_mokhtar@yahoo.fr

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References

- [1] Lock JM. Legumes of Africa: A Check List. Kew, England, Royal Botanic Gardens; 1989
- [2] Anon. Lost Crops of Africa. Washington: National Academy Press; 2006
- [3] Le Houerou HN. La végétation de la Tunisie steppique (1) (Structure, écologie, sociologie, répartition, évolution, utilisation, biomasse, productivité) (avec référence aux végétations analogues d'Algérie, de Libye et du Maroc). Annales de l'Institut National de la Recherche Agronomique de la Tunisie. 1969;**42**:622
- [4] Requena N, Pérez-Solis E, Azcón-Aguilar C, Jeffries P, Barea JM. Management of indigenous plant-microbe symbioses aids restoration of desertified ecosystems. Applied and Environmental Microbiology. 2001;**67**:495-498
- [5] Abdel-Ghaffar AS. Aspects of microbial activities and nitrogen fixation in Egyptian desert soils. Arid Soil Research and Rehabilitation. 1989;**3**:281-294
- [6] Wullstein LH. Evaluation and significance of associative dinitrogen fixation for arid soil rehabilitation. Arid Soil Research and Rehabilitation. 1989;**3**:259-265
- [7] Caucas V, Abril A. *Frankia* sp. infects *Atriplex cordobensis*-cross-inoculation assay and symbiotic efficiency. Phyton. 1996;**59**:103-110
- [8] Sayed WF, Wheeler CT, Zahran HH, Shoreit AAM. Effect of temperature and moisture on the survival and symbiotic effectiveness of *Frankia* spp. Biology and Fertility of Soils. 1997;**25**:349-353
- [9] Freiberg C, Fellay R, Bairoch A, Broughton WJ, Rosenthal A, Perret X. Molecular basis of symbiosis between rhizobium and legumes. Nature. 1997;**387**:394-401
- [10] Peoples MB, Ladha JK, Herridge DF. Enhancing legume N₂ fixation through plant and soil management. Plant and Soil. 1995;**174**:83-101
- [11] Ahmad MH, Rafique MU, McLaughlin W. Characterization of indigenous rhizobia from wild legumes. FEMS Microbiology Letters. 1984;**24**:197-203
- [12] Jha PK, Nair S, Gopinathan MC, Babu CR. Suitability of rhizobia-inoculated wild legumes *Argyrobolium flaccidum*, *Astragalus graveolens*, *Indigofera gangetica* and *Lespedeza stenocarpa* in providing a vegetational cover in an unreclaimed lime stone quarry. Plant and Soil. 1995;**177**:139-149
- [13] Sprent JI. Legume Nodulation: A Global Perspective. Oxford, UK: Wiley-Blackwell; 2009
- [14] Young ND, Debellé F, Oldroyd GE, Geurts R, Cannon SB, Udvardi MK, et al. The *Medicago* genome provides insight into the evolution of rhizobial symbioses. Nature. 2011;**480**:520-524. DOI: 10.1038/nature10625
- [15] Giraud E, Moulin L, Vallenet D, Barbe V, Cytryn E, Avarre JC, et al. Legumes symbioses: Absence of nod genes in photosynthetic bradyrhizobia. Science. 2007;**316**:1307-1312. DOI: 10.1126/science.1139548
- [16] Wang D, Yang SM, Tang F, Zhu HY. Symbiosis specificity in the legume—Rhizobial mutualism. Cellular Microbiology. 2012;**14**:334-342. DOI: 10.1111/j.1462-5822.2011.01736
- [17] Spaink HP, Wijffelman CA, Pees E, Okker RJH, Lugtenberg BJJ. Rhizobium nodulation gene *nodD* as a determinant

of host specificity. *Nature*. 1987;**328**: 337-340. DOI: 10.1038/328337a0

[18] Long SR. Genes and signals in the rhizobium-legume symbiosis. *Plant Physiology*. 2001;**125**:69-72. DOI: 10.1104/pp.125.1.69

[19] Sato S, Nakamura Y, Kaneko T, Asamizu E, Kato T, Nakao M, et al. Genome structure of the legume, *Lotus japonicus*. *DNA Research*. 2008;**15**: 227-239. DOI: 10.1093/dnares/dsn008

[20] Marx H, Minogue CE, Jayaraman D, Richards AL, Kwiecien NW, Sihapirani AF, et al. A proteomic atlas of the legume *Medicago truncatula* and its nitrogen-fixing endosymbiont *Sinorhizobium meliloti*. *Nature Biotechnology*. 2016;**34**:1198-1205. DOI: 10.1038/nbt.3681

[21] Tolin S, Arrigoni G, Moscattiello R, Masi A, Navazio L, Sablok G, et al. Quantitative analysis of the naringenin-inducible proteome in *Rhizobium leguminosarum* by isobaric tagging and mass spectrometry. *Proteomics*. 2013;**13**:1961-1972. DOI: 10.1002/pmic.201200472

[22] Čuklina J, Hahn J, Imakaev M, Omasits U, Förstner KU, Ljubimov N, et al. Genome-wide transcription start site mapping of *Bradyrhizobium japonicum* grown free-living or in symbiosis—A rich resource to identify new transcripts, proteins and to study gene regulation. *BMC Genomics*. 2016;**17**:302. DOI: 10.1186/s12864-016-2602-9

[23] Remigi P, Zhu J, Young JPW, Masson-Boivin C. Symbiosis within symbiosis: Evolving nitrogen-fixing legume symbionts. *Trends in Microbiology*. 2016;**24**:63-75. DOI: 10.1016/j.tim.2015.10.007

[24] Peix A, Ramirez-Bahena MH, Velazquez E, Bedmar EJ. Bacterial associations with legumes. *Critical Reviews in Plant Sciences*. 2015;**34**:17-42

[25] Pueppke SG, Broughton WJ. *Rhizobium* sp. strain NGR234 and *R. fredii* USDA257 share exceptionally broad, nested host ranges. *Molecular Plant-Microbe Interactions*. 1999;**12**:293-318

[26] Perret X, Staehelin C, Broughton WJ. Molecular basis of symbiotic promiscuity. *Microbiology and Molecular Biology Reviews*. 2000;**64**:180-201

[27] Rogel MA, Ormeno-Orrillo E, Martinez-Romero E. Symbiovars in rhizobia reflect bacterial adaptation to legumes. *Systematic and Applied Microbiology*. 2011;**34**:96-104

[28] Sprent JI, Ardley J, James EK. Tansley review: Biogeography of nodulated legumes and their nitrogen-fixing symbionts. *New Phytologist*. 2017;**215**:40-56. DOI: 10.1111/nph.14474

[29] Jordan DC. NOTES: Transfer of *Rhizobium japonicum* Buchanan 1980 to *Bradyrhizobium* gen. nov., a genus of slow-growing, root nodule bacteria from leguminous plants. *International Journal of Systematic and Evolutionary Microbiology*. 1982;**32**:136-139. DOI: 10.1099/00207713-32-1-136

[30] Djouadi S, Amrani S, Bouherama A, Nazhat-Ezzaman N, Aïd F. Nature des rhizobia associés à 15 espèces du genre *Lotus* en Algérie. *Botany*. 2017;**95**: 879-888. DOI: 10.1139/cjb-2017-0020

[31] Zakhia F, Jeder H, Domergue O, Willems A, Cleyet-Marel JC, Gillis M, et al. Characterization of wild legume nodulating bacteria (LNB) in the infra-arid zone of Tunisia. *Systematic and Applied Microbiology*. 2004;**27**:380-395. DOI: 10.1078/0723-2020-00273

[32] Amrani S, Nazhat-Ezzaman N, Bhatnagar T. Caractéristiques symbiotiques et génotypiques des Rhizobia associées à *Acacia saligna* (Labill.) Wendl. dans quelques

pépinières en Algérie. Acta Botanica Gallica. 2009;156:501-513

[33] Boukhatem ZF, Domergue O, Bekki A, Merabet C, Sekkour S, Bouazza F, et al. Symbiotic characterization and diversity of rhizobia associated with native and introduced acacias in arid and semi-arid regions in Algeria. FEMS Microbiology Ecology. 2012;80:534-547

[34] Fikri-Benbrahim K, Chraïbi M, Lebrazi S, Moumni M, Ismaili M. Phenotypic and genotypic diversity and symbiotic effectiveness of rhizobia isolated from *Acacia* sp. grown in Morocco. Journal of Agricultural Science and Technology. 2017;19:201-216

[35] Boulila F, Depret G, Boulila A, Belhadi D, Benallaoua S, Laguerre G. *Retama* species growing in different ecological-climatic areas of northeastern Algeria have a narrow range of rhizobia that form a novel phylogenetic clade within the *Bradyrhizobium* genus. Systematic and Applied Microbiology. 2009;32:245-255. DOI: 10.1016/j.syapm.2009.01.005

[36] Hannane FZ, Kacem M, Kaid-Harche M. Preliminary characterization of slow growing rhizobial strains isolated from *Retama monosperma* (L.) Boiss. root nodules from northwest coast of Algeria. African Journal of Biotechnology. 2016;15:854-867

[37] Guerrouj K, Perez-Valera E, Chahboune R, Abdelmoumen H, Bedmar EJ, El Idrissi MM. Identification of the rhizobial symbiont of *Astragalus globbiformis* in eastern Morocco as *Mesorhizobium camelthorni*. Antonie Van Leeuwenhoek. 2013;104:187-198

[38] Menna P, Barcellos FG, Hungria M. Phylogeny and taxonomy of a diverse collection of *Bradyrhizobium* strains based on multilocus sequence analysis of the 16S rRNA gene, ITS region and

glnII, *recA*, *atpD* and *dnaK* genes. International Journal of Systematic and Evolutionary Microbiology. 2009;59:2934-2950

[39] Chahboune R, Carro L, Peix A, Barrijal S, Velázquez E, Bedmar EJ. *Bradyrhizobium cytisi* sp. nov., isolated from effective nodules of *Cytisus villosus*. International Journal of Systematic and Evolutionary Microbiology. 2011;61:2922-2927

[40] Chahboune R, Carro L, Peix A, Ramirez-Bahena MH, Barrijal S, Velasquez E, et al. *Bradyrhizobium rifense* sp. nov. isolated from effective nodules of *Cytisus villosus* grown in the Moroccan Rif. Systematic and Applied Microbiology. 2012;35:302-305

[41] Ahnia H, Boulila F, Boulila A, Boucheffa K, Durán D, Bourebaba Y, et al. *Cytisus villosus* from northeastern Algeria is nodulated by genetically diverse *Bradyrhizobium* strains. Antonie Van Leeuwenhoek. 2014;105:1121-1129. DOI: 10.1007/s10482-014-0173-9

[42] Chahboune R, El Akhal MR, Arakrak A, Bakkal M, Laglaoui A, Pueyo JJ, et al. Characterization of bradyrhizobia isolated from root nodules of *Cytisus triflorus* in the rif occidental of morocco. In: Proceedings of the 15th International Nitrogen Fixation Congress and the 12th International Conference of the African Association for Biological Nitrogen Fixation. 2008. p. 155

[43] Bourebaba Y, Durán D, Boulila F, Ahnia H, Boulila A, Temprano F, et al. Diversity of *Bradyrhizobium* strains nodulating *Lupinus micranthus* on both sides of the Western Mediterranean: Algeria and Spain. Systematic and Applied Microbiology. 2016;39:266-274. DOI: 10.1016/j.syapm.2016.04.006

[44] Msaddak A, Durán D, Rejili M, Mars M, et al. Diverse bacteria affiliated with the genera *Microvirga*,

Phyllobacterium and *Bradyrhizobium* nodulate *Lupinus micranthus* growing in soils of Northern Tunisia. Applied and Environmental Microbiology. 2017;**83**(6). pii: e02820-16. DOI: 10.1128/AEM.02820-16

[45] Msaddak A, Rejili M, Durán D, Rey L, Palacios JM, Imperial J, et al. Definition of two new symbiovars, sv. *lupini* and sv. *mediterraneense*, within the genera *Bradyrhizobium* and *Phyllobacterium* efficiently nodulating *Lupinus micranthus* in Tunisia. Systematic and Applied Microbiology. 2018;**41**:487-493

[46] Jarvis BDW, van Berkum P, Chen WX, Nour SM, Fernandez MP, Cleyet-Marel JC, et al. Transfer of *Rhizobium loti*, *Rhizobium huakuii*, *Rhizobium ciceri*, *Rhizobium mediterraneum*, and *Rhizobium tianshanense* to *Mesorhizobium* gen. nov. International Journal of Systematic Bacteriology. 1997;**47**:895-898

[47] Mahdhi M, Houdheg N, Mahmoudi N, Msaadek A, Rejili M, Mars M. Characterization of rhizobial bacteria nodulating *Astragalus corrugatus* and *Hippocrepis areolata* in Tunisian arid soils. Polish Journal of Microbiology. 2016;**65**:331-339

[48] Ourarhi M, Abdelmoumen H, Guerrouj K, Benata H, Muresu R, Squartini A, et al. *Colutea arborescens* is nodulated by diverse rhizobia in Eastern Morocco. Archives of Microbiology. 2011;**193**:115-124. DOI: 10.1007/s00203-010-0650-0

[49] Rejili M, Lorite MJ, Mahdhi M, Pinilla JS, Ferchichi A, Mars M. Genetic diversity of rhizobial populations recovered from three *Lotus* species cultivated in the infra-arid Tunisian soils. Progress in Natural Science. 2009;**19**:1079-1087

[50] Rejili M, Mahdhi M, Fterich A, Dhaoui S, Guefrachi I, Abdeddayem R,

et al. Symbiotic nitrogen fixation of wild legumes in Tunisia: Soil fertility dynamics, field nodulation and nodules effectiveness. Agriculture, Ecosystems and Environment. 2012;**157**:60-69. DOI: 10.1016/j.pnsc.2009.02.003

[51] Rejili M, Mahdhi M, Domínguez-Núñez JA, Mars M. The phenotypic, phylogenetic and symbiotic characterization of rhizobia nodulating *Lotus* sp. in Tunisian arid soils. Annales de Microbiologie. 2013;**64**:355-362

[52] Roba M, Willems A, Le Quéré A, Maynaud G, Pervent M, et al. *Mesorhizobium delmotii* and *Mesorhizobium prunaredense* are two new species containing rhizobial strains within the symbiovar *anthyllidis*. Systematic and Applied Microbiology. 2017;**40**:135-143

[53] Chaich K, Bekki A, Bouras N, Holtz MD, Soussou S, Maure L, et al. Rhizobial diversity associated with the spontaneous legume *Genista saharae* in the northeastern Algerian Sahara. Symbiosis. 2017;**71**(2):111-120

[54] Fterich A, Mahdhi M, Caviedes MA, Pajuelo E, Rivas R, Rodriguez-Llorente ID, et al. Characterization of root-nodulating bacteria associated to *Prosopis farcta* growing in the arid regions of Tunisia. Archives of Microbiology. 2011;**193**:385-397

[55] Young JM. Renaming of *Agrobacterium larrymoorei* Bouzar and Jones 2001 as *Rhizobium larrymoorei* (Bouzar and Jones 2001) comb. nov. International Journal of Systematic and Evolutionary Microbiology. 2004;**54**:149

[56] Mahdhi M, Mars M. Genotypic diversity of rhizobia isolated from *Retama raetam* in arid regions of Tunisia. Annales de Microbiologie. 2006;**56**:305-311

[57] Mahdhi M, Nzoué A, Gueye F, Merabet C, de Lajudie P, Mars M. Phenotypic and genotypic diversity

of *Genista saharae* microsymbionts from the infra-arid region of Tunisia. Letters in Applied Microbiology. 2007;**54**:604-609

[58] Mahdhi M, de Lajudie P, Mars M. Phylogenetic and symbiotic characterization of rhizobial bacteria nodulating *Argyrolobium uniflorum* in Tunisian arid soils. Canadian Journal of Microbiology. 2008;**54**:209-217

[59] Abdelmoumen H, Filali Maltout A, Neyra M, Belabed A, El Idrissi MM. Effects of high salts concentrations on the growth of rhizobia and responses to added osmotica. Journal of Applied Microbiology. 1999;**86**:889-898

[60] Bouchiba Z, Boukhatem ZF, Ighilhariz Z, Derkaoui N, Bekki A. Diversity of nodular bacteria of *Scorpiurus muricatus* in western Algeria and their impact on plant growth. Canadian Journal of Microbiology. 2017;**63**:450-463. DOI: 10.1139/cjm-2016-0493

[61] Merabet C, Bekki A, Benrabah N, Bey M, Bouchentouf L, Ameziane H, et al. Distribution of *Medicago* species and their microsymbionts in a saline region of Algeria. Arid Land Research and Management. 2006;**20**:219-231

[62] Sebbane N, Sahnoune M, Zakhia F, Willems A, Benallaoua S, de Lajudie P. Phenotypical and genotypical characteristics of root-nodulating bacteria isolated from annual *Medicago* spp. in Soummam Valley (Algeria). Letters in Applied Microbiology. 2006;**42**:235-241

[63] Mahdhi M, Fterich A, Rejili M, Rodriguez-Llorente ID, Mars M. Legume-Nodulating bacteria (LNB) from three pasture legumes (*Vicia sativa*, *Trigonella maritima* and *Hedysarum spinosissimum*) in Tunisia. Annales de Microbiologie. 2012;**62**:61-68

[64] Fitouri SD, Trabelsi D, Saïdi S, Zribi K, Ben Jeddi F, Mhamdi R. Diversity

of rhizobia nodulating sulla (*Hedysarum coronarium* L.) and selection of inoculant strains for semi-arid Tunisia. Annales de Microbiologie. 2012;**62**:77-84. DOI: 10.1007/s13213-011-0229-22012

[65] Ezzakkioui F, El Mourabit N, Chahboune R, Castellano-Hinojosa A, Bedmar EJ, Barrijal S. Phenotypic and genetic characterization of rhizobia isolated from *Hedysarum flexuosum* in northwest region of Morocco. Journal of Basic Microbiology. 2015;**55**:830-837. DOI: 10.1002/jobm.2014007

[66] Balkwill DL. Ensifer. In: Bergey's Manual of Systematics of Archaea and Bacteria. Hoboken, New Jersey-USA: John Wiley & Sons, Ltd; 2005

[67] Willems A, Fernandez-Lopez M, Munoz-Adelantado E, Goris J, et al. Description of new *Ensifer* strains from nodules and proposal to transfer *Ensifer adhaerens* Casida 1982 to *Sinorhizobium* as *Sinorhizobium adhaerens* comb. nov. request for an opinion. International Journal of Systematic and Evolutionary Microbiology. 2003;**53**:1207-1217

[68] Judicial Commission. Opinion 84 – The genus name *Sinorhizobium* Chen et al. 1988 is a later synonym of *Ensifer* Casida 1982 and is not conserved over the latter genus name, and the species name '*Sinorhizobium adhaerens*' is not validly published. International Journal of Systematic and Evolutionary Microbiology. 2008;**58**:1973

[69] Merabet C, Martens M, Mahdhi M, Zakhia F, et al. Multilocus sequence analysis of root nodule isolates from *Lotus arabicus* (Senegal), *Lotus creticus*, *Argyrolobium uniflorum* and *Medicago sativa* (Tunisia) and description of *Ensifer numidicus* sp. nov. and *Ensifer garamanticus* sp. nov. International Journal of Systematic and Evolutionary Microbiology. 2010;**60**:664-674

[70] Khbaya B, Neyra M, Normand P, Zerhari K, Filali-Maltouf A. Genetic

diversity and phylogeny of rhizobia that nodulate *Acacia* spp. in Morocco assessed by analysis of rRNA genes. *Applied and Environmental Microbiology*. 1998;**64**:4912-4917

[71] Fterich A, Mahdhi M, Mars M. Impact of grazing on soil microbial communities along a chronosequence of *Acacia tortilis* subsp. *raddiana* in arid soils in Tunisia. *European Journal of Soil Biology*. 2012;**50**:56-63

[72] Sakrouhi I, Belfquih M, Sbabou L, Moulin P, Bena G, Filali-Maltouf A, et al. Recovery of symbiotic nitrogen fixing acacia rhizobia from Merzouga Desert sand dunes in south East Morocco—Identification of a probable new species of *Ensifer* adapted to stressed environments. *Systematic and Applied Microbiology*. 2016;**39**:22-31

[73] Ben Romdhane S, Nasr H, Samba-Mbaye R, Neyra M, Ghorbal MH. Diversity of *Acacia tortilis* rhizobia revealed by PCR/RFLP on crushed root nodules in Tunisia. *Annals of Microbiology*. 2005;**55**:249-258

[74] Jebara M, Drevon JJ, Aouani ME. Effects of hydroponic culture system and NaCl on interactions between common bean lines and native rhizobia from Tunisian soils. *Agronomie*. 2001;**21**:601-605

[75] Zribi K, Mhamdi R, Huguet T, Aouani ME. Distribution and genetic diversity of rhizobia nodulating natural populations of *Medicago truncatula* in Tunisian soils. *Soil Biology and Biochemistry*. 2004;**36**:903-908. DOI: 10.1016/j.soilbio.2004.02.003

[76] Badri M, Ilahi H, Huguet T, Aouani ME. Quantitative and molecular genetic variation in sympatric populations of *Medicago laciniata* and *M. truncatula* (Fabaceae): Relationships with eco-geographical factors. *Genetical Research*. 2007;**89**:107-122

[77] Elboutahiri N, Thami-Alami I, Udupa SM. Phenotypic and genetic diversity in *Sinorhizobium meliloti* and *S. medicae* from drought and salt affected regions of Morocco. *BMC Microbiology*. 2015;**10**:15. DOI: 10.1186/1471-2180-10-15

[78] Baba Arbi S, Cheriet D, Chekireb D, Ouarts A. Caractérisation phénotypique et génotypique des rhizobia symbiotiques des légumineuses spontanées *Melilotus indicus* et *Medicago littoralis* des palmeraies de la région de Touggourt en Algérie. In: Conférence: Journées Internationales de Biotechnologie 2014, at Hammamet, Tunisie. Vol. 2014

[79] Guerrouj K, Bouterfas M, Abdelmoumen H, Missbah El Idrissi M. Diversity of bacteria nodulating *Medicago arborea* in the northeast area of Morocco. *Chiang Mai Journal of Science*. 2016;**43**:440-451

[80] Mousavi SA, Österman J, Wahlberg N, Nesme X, Lavire C, Vial L, et al. Phylogeny of the *Rhizobium* clade supports the delineation of *Neorhizobium* gen. nov. *Systematic and Applied Microbiology*. 2014;**37**:208-215

[81] Flores-Felix JD, Carro L, Velasquez E, Valverde A, Cerda Castillo E, Garcia-Fraile P, et al. *Phyllobacterium endophyticum* sp. nov., isolated from nodules of *Phaseolus vulgaris*. *International Journal of Systematic and Evolutionary Microbiology*. 2013;**63**:821-826

[82] Ardley J, O'Hara G, Reeve W, Yates R, Dilworth M, Tiwari R, et al. Root nodule bacteria isolated from South African *Lotononis bainesii*, *L. listii* and *L. solitudinis* are species of *Methylobacterium* that are unable to utilize methanol. *Archives of Microbiology*. 2009;**191**:311-318

[83] Andam CP, Parker MA. Novel alphaproteobacterial root nodule

symbiont associated with *Lupinus texensis*. Applied and Environmental Microbiology. 2007;73:5687-5691. DOI: 10.1128/AEM.01413-07

[84] Reeve W, Chain P, O'Hara G, Ardley J, Nandesena K, Bräu L, et al. Complete genome sequence of the *Medicago* microsymbiont *Ensifer* (*Sinorhizobium*) *medicae* strain WSM419. Standards in Genomic Sciences. 2010;2:77

[85] Tinick MJ, Hadobas PA. Nodulation of *Trifolium repens* with modified *Bradyrhizobium* and the nodulation of *Parasponia* with *Rhizobium leguminosarum* biovar *trifolii* 1990a. Plant and Soil. 1990;125:49-61

[86] Mahdhi M, Nzoué A, de Lajudie P, Mars M. Characterization of root-nodulating bacteria on *Retama raetam* in arid Tunisian soils. Progress in Natural Science. 2008;18:43-49