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COMMENTARY

Legumes of the Thar desert and their nitrogen fixing *Ensifer* symbionts

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Abstract
Background Nodulated legumes form a large component of the flora of desert regions, but comparatively few studies have characterised their symbiotic interactions, or their rhizobial microsymbionts. The Thar desert is a sub-tropical desert with infertile, alkaline soils in the northwestern part of the Indian subcontinent, which is home to a large number of legumes that provide food and fuel, or are of medicinal importance to the local people. In the latest in a series of studies on the nodulated legumes of the Thar desert and their associated rhizobia, Sankhla et al. (2016), this issue, have characterised strains of *Ensifer* rhizobia isolated from nodules of the native mimosoid legume shrub *Vachellia* (*Acacia*) *jacquemontii.*
Scope These studies suggest that *Ensifer* rhizobia are the dominant microsymbionts of legumes native to the Thar desert and are particularly well-adapted to the arid conditions and alkaline soils found there. The *Ensifer* strains that nodulate *V. jacquemontii* may constitute a novel species, based on 16S rRNA and Multi Locus Sequence Analysis. Symbiotic adaptation of these rhizobia to local host legumes has involved horizontal gene transfer of symbiosis genes.
Conclusions These findings provide further insights into the evolution of legume-rhizobia symbioses and how plants and rhizobia are adapted to and can survive in stressful conditions.

Keywords Legumes · Rhizobia · *Vachellia* (*Acacia*) *jacquemontii* · *Ensifer* · Biogeography · Thar desert · Alkaline soil

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The Thar Desert, a 200, 000 km^2 expanse of arid and semi-arid sand dunes and plains that extends from India into Pakistan, is the world's 18th largest desert. It is classified as a sub-tropical desert, characterised by low rainfall (50 to 300 mm), high temperatures and infertile, alkaline soils (Bhandari 1990). It is also remarkable for being the most densely populated desert in the world, home to over 17 million people and 23 million livestock (Khan et al. 2003; Singh et al. 2006). The desert flora includes a large number of plants that are of economic or medicinal importance and 45 species that are classified as rare or endangered. Numerically, legumes (Leguminosae) are the most dominant family in terms of both genera and species (Bhandari 1990), and play a number of important economic and ecological roles, such as providing food for people and livestock, being sources of firewood, timber, honey or medicine, and maintaining soil fertility.

Most legumes are able to form dinitrogen (N_2)–fixing symbioses with a group of phylogenetically diverse soil bacteria, belonging to the Alpha- or Betaproteobacteria, that are collectively known as rhizobia. A molecular dialogue between the host plant and the rhizobia governs symbiotic specificity, infection and the formation of a nodule, within which the rhizobia are surrounded by a plant-derived membrane (the symbiosome) and differentiate into their N_2-fixing bacteroid form (Oldroyd et al. 2011). The study of the symbiotic interactions between legumes and their cognate rhizobia has been, and continues to be, dominated by research on agriculturally important legumes such as soybean, or lucerne (alfalfa), or the model legumes *Medicago truncatula* and *Lotus japonicus* (Jones et al. 2007; Madsen et al. 2010). However, various studies on the interactions of wild legumes with rhizobia within their native range are now providing us with insights into the biogeography of rhizobia and the patterns of their symbiotic associations with their legume partners. In this regard, the paper in this issue by Sankhla et al. (2016) is the latest in a series of studies (Gehlot et al. 2012; Gehlot et al. 2013) that describe the nodulated legumes of the Thar desert and characterise their rhizobial microsymbionts.

These studies provide valuable insights, not only into the ecology of legumes and rhizobia in the Thar desert, but also into the evolution of symbioses. The current study describes nodulation in the native mimosoid legume shrub *Vachellia* (*Acacia*) *jacquemontii* and characterises its N_2-fixing rhizobial microsymbionts. Firstly, Sankhla and colleagues have noted that nodulation was observed in nearly all plants sampled, although reduced in numbers in the hyper-arid sites. This accords with previous observations that mimosoid legume shrubs in seasonally dry environments cease nodulation and/or shed nodules during dry periods (Sprent 2009; dos Reis et al. 2010; Bontemps et al. 2016). The corky layers in the nodule cortex, with phenolic/tannin containing cells, are typical of mimosoid nodules, while the additional "waterproof" corky layers on the nodule tip may be an adaptation to arid soils. The ultrastructure of the indeterminate *V. jacquemontii* nodules is remarkably consistent with that observed in other mimosoid legumes, regardless of their phylogenetic placement or microsymbiont type (Gehlot et al. 2013; Cordero et al. 2016): the central nodule tissue contains a mix of infected and uninfected cells and up to four non-swollen bacteroids within each symbiosome. This uniformity contrasts with the situation in papilionoid legumes, which have a wide range of nodule phenotypes (Sprent 2009).

Secondly, all 73 isolates from *V. jacquemontii* nodules were found to belong to a number of genotypes within the genus *Ensifer* (*Sinorhizobium*). Previous studies of rhizobia isolated from nodulated legumes of the Thar desert have also found a preponderance of *Ensifer* strains, suggesting that they are the dominant microsymbionts of legumes native to this system (Gehlot et al. 2012; Gehlot et al. 2013). Indeed, various studies of rhizobia isolated from legumes growing in arid climates and/or alkaline soils have also found that *Ensifer* genotypes predominate in these conditions (Yates et al. 2004; Wolde-Meskel et al. 2005; Romdhane et al. 2006; Han et al. 2009; Xu et al. 2013; Bontemps et al. 2016). As *Ensifer* strains appear to be better adapted to the arid climate and alkaline edaphic conditions of the Thar desert, it is likely that they are also the predominant rhizobial genotypes existing as saprophytes within the soil or plant rhizospheres, and thus available for selection by the legume host. In contrast, in another mimosoid legume, the rhizobia isolated from *Mimosa* spp. growing in the acidic, infertile soils of their main centre of diversity in central Brazil are overwhelmingly nodulated by rhizobial species of the acid tolerant Betaproteobacterial genus *Burkholderia* (Bontemps et al. 2010; Stopnisek et al. 2014). Perhaps the radiation of legumes from their original centres of diversity may eventually involve a change of microsymbiont to one more adapted to the local edaphic conditions, although this would appear to favour symbiotic generalists, rather than specialists. This is neatly illustrated by Gehlot and colleagues' study of *Mimosa* spp. growing in the Thar desert, which showed that the native species *M. hamata* was nodulated by species of *Ensifer*, whereas the invasive *M. pudica* was nodulated by strains of *Burkholderia*, which it appeared to have carried with it from South America (Gehlot et al. 2013).

Finally, Sankhla and colleagues have demonstrated that most of the *Ensifer* strains of the Thar Desert are new lineages within the Old World *E. saheli*, *E. kostiensis* and *E. arboris* groups that have been isolated from African legumes (de Lajudie et al. 1994; Nick et al. 1999). The *Ensifer* strains that nodulate *V. jacquemontii* may constitute a novel species, based on 16S rRNA and Multi Locus Sequence Analysis. Most of the *V. jacquemontii Ensifer* isolates were able to nodulate a wide diversity of the woody mimosoid legumes native to the Thar desert. Furthermore, phylogenies of the symbiosis-related *nod*A and *nifH* genes were incongruent with the 16S rRNA phylogeny, suggesting that the symbiotic adaptation of the Thar desert *Ensifer* strains to local host legumes has involved horizontal gene transfer of symbiosis genes, which appears to be a not uncommon occurrence among rhizobia (Rogel et al. 2011).

The need to respond to the current threats of climate change and increasing desertification should bring a greater interest in understanding how plants can survive in harsh environments. Nodulated legumes, which appear to be particularly favoured by arid conditions (Pellegrini et al. 2016), can provide food and fuel, and maintain soil fertility. They are obvious targets for renewed research efforts into integrating them into agricultural and agroforestry systems in marginal lands, or mining them for the traits that allow them to survive in arid conditions (Sprent and Gehlot 2010). We also need a greater understanding of how their rhizobial microsymbionts can survive in these environments, and the basis of the rhizobial ability to form N_2-fixing symbioses with plants under these conditions. The Genomic Encyclopedia of Bacteria and Archaea – Root Nodule Bacteria (GEBA-RNB) sequencing project (Reeve et al. 2015) was designed to provide such perspectives on the evolution, ecology and biogeography of legume-RNB symbioses. The genomes of two of the rhizobial strains isolated from Thar desert legumes have now been sequenced as part of the GEBA-RNB project (Tak et al. 2013; Gehlot et al. 2016) and will greatly assist these research efforts.

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