Current knowledge and future prospects of lima bean (*Phaseolus lunatus*)-rhizobia symbiosis

Conocimiento actual y perspectivas de futuro de la simbiosis frijol de lima (*Phaseolus lunatus*)-rizobia

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Review

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Abstract

Lima bean (*Phaseolus lunatus)* is an important species of the genus *Phaseolus* for human consumption in tropical regions. The seeds are important source of protein for people from South America, Africa and Mexico. In addition, as a legume plant, lima bean presents the ability to perform the biological nitrogen fixation (BNF) through the symbiosis with nitrogen-fixing bacteria. The studies about diversity and efficiency of lima bean-rhizobia symbiosis have increased worldwide, mainly in Latin America. These studies have shown *Bradyrhizobium* and *Rhizobium* as the main symbionts, although *Sinorhizobium*, *Mesorhizobium* and *Allorhizobium* have been found associated with lima bean. Also, there is a large variation in the efficiency of N fixation by the current isolates of rhizobia and some rhizobia have presented high capability for fixing N. This review aims to explore the studies about diversity and efficiency of rhizobia in symbiosis with lima bean.

Keywords
BNF-efficiency • *Phaseolus* • rhizobia diversity

Resumen

El frijol de lima (*Phaseolus lunatus*) es una especie importante del género *Phaseolus* para consumo humano en regiones tropicales. Las semillas son una fuente importante de proteínas para las personas de América del Sur, África y México. Además, como leguminosas, el frijol de lima presenta la capacidad de realizar la fijación biológica de nitrógeno (BNF) a través de la simbiosis con bacterias fijadoras de nitrógeno. Los estudios sobre la diversidad y la eficiencia de la simbiosis de frijol de lima-rizobio han aumentado en todo el mundo, principalmente en América Latina. Estos estudios han demostrado que Bradyrhizobium y Rhizobium son los principales simbiontes, aunque Sinorhizobium, Mesorhizobium y Allorhizobium se han asociado al frijol de lima. Además, existe una gran variación en la eficacia de la fijación de N por los aislamientos actuales de rizobios y algunos rizobios han presentado una alta capacidad para reparar N. Esta revisión tiene como objetivo explorar los estudios sobre la diversidad y la eficacia de rizobios en simbiosis con frijol lima.

Palabras clave
BNF-eficiencia • *Phaseolus* • diversidad de rizobios

Introduction

Legume plants belonging to the genus *Phaseolus* present economic importance worldwide. This genus comprises five species: *P. vulgaris* L., *P. lunatus* L., *P. coccineus* L., *P. acutifolius* A. Gray and *P. polyanthus* Greeman (14). Lima bean (*P. lunatus* L.) is considered the second most cultivated species from the genus *Phaseolus*, being an important species of plant for humans in tropical regions (11, 17, 23).

This legume is originated from Perú and was domesticated in the Andean Mountains, specifically in southern Ecuador-northwestern Perú, and central-western Mexico (25). The study of Mota-Aldana *et al.* (2010) also identified three main varieties: "Big Lima" (big seeds) domesticated in the Andean Mountains; "Sieva" and "Potato" (small seeds) originated in central-western Mexico. Lima bean is predominantly autogamous, although it has outcrossing rates up to 48% (6). It has been characterized by an indeterminate growth habit, a prolonged flowering period, and production of a large number of pods (37).

Lima bean is considered an important source of protein for people from South America, Africa, and Mexico. Interestingly, this crop is economically important in some regions of United States. For example, California has about 24,000 acres of lima bean with a value of about $32 million in 2017 (35). The seeds present high content of protein (210-260 $g \cdot kg^{-1}$) and carbohydrate (550-640 $g \cdot kg^{-1}$), low fat (10-23 $g \cdot kg^{-1}$) levels and high fiber levels (32-68 $g \cdot kg^{-1}$), high levels of the minerals K, Zn, Ca, and Fe, and low levels of Na and P (7).

It is also a relevant plant for Brazil, mainly in the Northeast region, where it is used as an alternative food source. Currently, Brazil produces approximately 14,951 t of lima bean from an area of about 37,521 ha. Since the Northeast region presents high annual temperature and drought periods, lima bean is adapted to these conditions and presents rusticity and tolerance to long and dry periods (5).

On the other hand, lima bean also presents the ability to associate symbiotically with nitrogen-fixing bacteria, commonly known as rhizobia, and therefore can perform the process of biological nitrogen fixation (BNF) (33). Since this plant needs about 100 kg N to produce 1.5 t of seeds (18), the BNF could be an ecological and economical alternative for supplying N to the plant.

Biological nitrogen fixation in lima bean

BNF is an important source of nitrogen (N) to the plants, which is important for plant growth. It has suggested that after photosynthesis, the process performed by plants to produce the energy necessary for their survival, BNF is regarded as the second most important biological process on the earth (http://cnx.org/content/m47338/latest/). This process occurs through of a symbiotic system between legumes and rhizobia (36), and the fixed N_2 represents a renewable source of N within agriculture. The amount of N fixed by plants is ranged from 100 to 900 kg ha^{-1} $year^{-1}$. For example, in soybean, an important crop worldwide, the contribution of N derived from the atmosphere via BNF represents 0 to 337 kg N ha^{-1} (9).

The symbiosis rhizobia-legume occurs in root nodules that are established by legume in response to appropriate rhizobia (2, 33). Thus, the nodulation is usually a host-specific interaction where some specific bacteria infect a limited range of legumes and the N-fixing nodules are formed as a consequence of this interaction (30). Rhizobia able to nodulate and fix N_2 in legumes comprise bacteria distributed among the genera *Agrobacterium, Allorhizobium, Azorhizobium, Bradyrhizobium, Burkholderia, Cupriavidus, Devosia, Herbaspirrilum, Mesorhizobium, Methylobacterium, Ochrobactrum, Phyllobacterium, Rhizobium, Shinella* and *Sinorhizobium* (8).

The success of symbiosis depends on the availability of compatible rhizobia to the specific legumes. Considering the genus *Phaseolus*, the studies have presented information about nodulation and BNF in common bean (*P. vulgaris*), which form symbiotic associations with several species from the genus *Rhizobium* (20, 21). There are variations among the efficiency of BNF in common bean. Recently, Moreira *et al.* (2017) evaluated the contribution of rhizobia isolates on N derived from the atmosphere, via BNF, to *P. vulgaris* and found a range of 509 to 1037 mg plant^{-1} which represented 46% to 75% of the total N found in the plants. For lima bean, the studies about symbiosis with rhizobia have shown a miscellaneous of genera able to nodulate this legume with different efficiencies. In this way, studies about the diversity of rhizobia which present ability for associating with lima bean are increasing in the last years. Thus, this review aims to explore the studies about diversity and efficiency of rhizobia in symbiosis with lima bean.

Diversity of lima bean-nodulating rhizobia

Rhizobia are common inhabitants of diverse soil types and the number of cells can substantially differ from a few cells to 10^5 bacteria per gram of soil (13). The wide geographical distribution of rhizobia may be related to the large genetic diversity among these bacteria (22). The knowledge about diversity of rhizobia provides information on host preferences, predominance of isolates, genetic structure and also source of efficient strains for using as inoculant (21).

The majority of legume-nodulating rhizobia belong to α-proteobacteria group that include *Rhizobium, Sinorhizobium, Azorhizobium, Bradyrhizobium, Allorhizobium, Mesorhizobium, Bradyrhizobium* and *Methylobacterium* (16). Usually, each genus is associated and nodulates with specific legumes. For example, *Rhizobium* is associated with common bean (*Phaseolus vulgaris* L.), while *Bradyrhizobium* nodulates soybean (*Glycine max* L. Merrill) and cowpea (*Vigna unguiculata* L.) (15).

Previous old host-based classification scheme included symbionts of lima bean in the same group of rhizobia associated with slow-growing cowpea (*Vigna unguiculata)* (29). This group was a diverse assemblage of strains that were later included in the genus *Bradyrhizobium* (1, 34). In these studies, the rhizobial isolates associated with lima bean were obtained from areas where this plant species is not native and the studies have only focused on morphological, physiological and symbiotic characteristics. Thus, the limitation of these methods did not address properly the existing diversity.

Currently, studies have been conducted with molecular tools that provide the possibility to identify taxonomic groups of rhizobia in soils and have shown several genera with ability to associate with lima bean (table 1, page 284). Indeed, molecular tools have identified a diverse group of rhizobia-nodulating lima bean comprising bacteria belonging to *Bradyrhizobium*, *Rhizobium*, *Sinorhizobium*, *Mesorhizobium* and *Allorhizobium* (4, 14, 26, 28, 32).

Table 1. Rhizobia genera isolated from lima bean nodules.
Tabla 1. Géneros de Rhizobia aislados de nódulos de frijol de lima.

Genera	Location	Method	Reference
Bradyrhizobium	USA	Morphology and physiology	Thies *et al.*, 1991
B. yuanmingense *Bradyrhizobium sp*	Perú	16S rDNA and *dnaK*, *nifH*, and *nodB* genes	Ormeño-Orillo *et. al.*, 2006
Bradyrhizobium	Mexico	16S rDNA and recA, nodZ and nifH genes.	Lopez-Lopez *et al.*, 2013
Bradyrhizobium	Perú	16S rDNA and recA, atpD, glnII, dnaK and gyrB genes.	Duran *et al.*, 2014
Bradyrhizobium, *Mesorhizobium, Rhizobium*	Brazil	endonucleases MboI, HaeIII, and NheI	Santos *et al.*, 2011
B. yuanyrense *B. liaoningense* *B. paxllaeri* *B. icense*	Perú	16S rRNA	Matsubara and Zúñiga-Dávila, 2015
R. mesosinicum *R. alamii*	Perú	16S rRNA	Matsubara and Zuñiga-Dávila, 2015
Rhizobium *Bradyrhizobium,* *Allorhizobium*	Brazil	16S rDNA	Araujo *et al.*, 2015

The majority of studies about the diversity of rhizobia in lima bean have assessed a relative wide collection from geographic locations including Perú, Mexico and Brazil (4, 14, 19, 23, 26, 32). Perú is considered the center of diversity of this plant species and the first study on the diversity of rhizobia, by using molecular tools, has characterized some symbionts of lima bean (26). Thus, Ormeño-Orrillo *et al.* (2017) evaluated the molecular diversity of rhizobial isolates associated with lima bean and found divergent bradyrhizobial lineages (*B. yuanmingense* and *Bradyrhizobium* sp.) according to Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) of the *rpoB, dnaK*, *nifH* and *nodB* genes and sequencing of the 16S rDNA. This finding could confirm that *Bradyrhizobium* is the main genus effectively associated lima bean. Indeed, the study conducted later by Duran *et al.* (13), by using a polyphasic approach, confirmed *Bradyrhizobium*-nodulating lima bean in Perú. However, the authors found two different lineages (*B. paxllaeri* sp. and *B. icense*) those described by Ormeno-Orrillo *et al.* (2006). The hypothesis that *Bradyrhizobium* is the most important genus found in Perú was addressed by Matsubara and Zuñiga-Dávila (2015) who evaluated rhizobia associated with lima bean in Perú and found species belonging to *Bradyrhizobium* genus (*B. yuanyrense, B. liaoningense, B. paxllaeri,* and *B. icense*). However, this study also reported the genus *Rhizobium* (*R. mesosinicum* and *R. alamii*). Thus, these studies showed that there is a high diversity of rhizobia associated with lima bean in Perú and it indicates a relative promiscuity of lima bean in contrast to previous statements that this plant species has a restricted symbiont (28).

As an important center of domestication (25), Mexico also has a high diversity of rhizobia able to nodulate lima bean. The first study about diversity of rhizobia-nodulating lima bean in Mexico was conducted by Lopez-Lopez *et al.* (2013) who sequenced *rpoB*, *recA*, *nodZ*, and *nifH* genes of the rhizobia and found isolates described as *Bradyrhizobium*. It was the first report of nodule bacteria from lima bean in Mesoamerican region (center of origin and domestication) and it confirms *Bradyrhizobium* as the main bacteria associated with lima bean. Therefore, *Bradyrhizobium* seems to be the most abundant genus nodulating lima bean in Perú and Mexico.

Brazil is an important region of dispersion of lima bean and the studies have shown a high diversity of rhizobia associated with this plant species. Thus, a study about genetic diversity of native rhizobia associated with lima bean in Brazil was conducted by Santos *et al.* (2011) and shows a broad spectrum of rhizobial groups. In this study, rhizobia isolates were obtained and placed into groups based on divergence in their morphological, physiological and genetic traits. The restriction patterns obtained with endonucleases MboI, HaeIII, and NheI showed sufficient variability and identified isolates belonging to the genera *Bradyrhizobium*, *Mesorhizobium* and *Rhizobium*. Later, Araujo *et al*. 2015, sequenced the 16S rDNA of these isolates and found species belonged to the genus *Bradyrhizobium*, *Sinorhizobium* and *Rhizobium.* These results confirm that lima bean may be associated by diverse rhizobia species and also showed different groups than those reported in Perú and Mexico. Indeed, Araújo *et al.* (2015) found 14 isolates of rhizobia genetically different that those observed in Perú by Ormeño-Orillo *et al.* (2006). The isolates found by Araujo *et al.* (2016) were classified as *Rhizobium, Bradyrhizobium* and *Allorhizobium.*

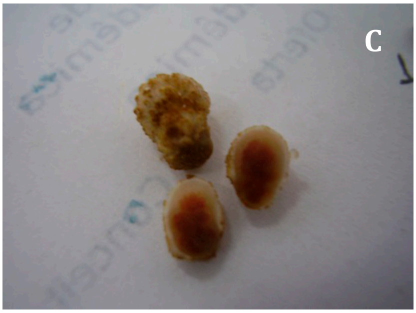
Efficiency of rhizobia on BNF in lima bean

Studies about nodulation and efficiency of BNF have increased in the last ten years and the main objective is to evaluate suitable strains for recommendation of inoculation. These studies have focused on the evaluation of rhizobia in different genotypes of lima bean (3, 10, 11). Indeed, the first study about nodulation in lima bean evaluated different genotypes (12). The main question was to determine if the nodule formation coincides with plant development and they found a close relationship between plant and nodule growth. Later, Santos *et al.* (2009) evaluated the ontogeny of nodulation in lima bean and found that the highest nodule number and biomass occurred between 45 and 60 days after plant emergence, which correspond to the flowering period of the plant. Additionally, Santos *et al.* (2009) found red colored nodules as an indication of leghemoglobin (photo 1, page 286). However, the confirmation that the rhizobia had high efficiency in fixing N in lima bean was reported by Antunes *et al.* (2004) who evaluated the symbiotic effectiveness of 17 rhizobial isolates associated with lima bean and compared with two strains of reference CIAT 899 and NGR 234. They found eight isolates with higher N accumulation and N_2-fixation efficiency compared with the strains CIAT 899 and NGR 234. Rhizobia isolated from different legume species seem to present ability for nodulating and fixing N in lima bean. Thus, Costa *et al.* (2017) evaluated rhizobia isolates from nodules of *Vigna unguiculata*, *Campsiandra surinamensis*, *Inga sp*. and *Swartzia sp.* on lima bean and found isolates with high efficiency in N fixation. Therefore, the prospection of rhizobia able to nodulate lima bean should be also done in different legume species.

As reported above, the main genus found in association with lima bean is *Bradyrhizobium*, although *Rhizobium* and other genera are able to associated with this plant species. Thus, in order to evaluate the efficiency of *Bradyrhizobium* and *Rhizobium* on nodulation and efficiency of BNF in lima bean, Costa Neto *et al.* (2017) found *Bradyrhizobium* more efficient than *Rhizobium* in establishing the symbiotic association with lima bean varieties. These authors also found isolates with high efficiency as compared with the other symbiotic pairs. Thus, the studies are in advance to find suitable and potential strains for inoculation in lima bean.

Future prospects

Lima bean is an important legume in tropical regions and the studies have shown a great diversity of rhizobia associated with this plant species. Also, there is a large variation in BNF efficiency observed in the current isolates of rhizobia. Therefore, further studies have to focus on: a) classification of rhizobia associated with lima bean in different regions; b) selection of specific and efficient strains for using as inoculant; c) improvement of N fixation ability by native rhizobia and adapted to different regions; d) selection of lima bean genotypes combined with high efficient strains; e) implementation of programs of lima bean breeding and management in Perú, Mexico and Brazil; f) analysis of the advantages of an adequate symbiosis for improving the content of protein and better quality of the nutritional offer for human nutrition.



(A) Nodules in the roots; **(B)** separated nodules; **(C)** Leg-hemoglobin pigment in nodule of lima bean (Source: Jadson E. L. Antunes)

(A) Nódulos en las raíces; **(B)** nódulos separados; **(C)** Pigmento de hemoglobina en nódulo de frijol de lima (Fuente: Jadson E. L. Antunes).

Photo 1. Nodules in lima bean roots at 45 days after plant emergence.

Foto 1. Nódulos en raíces de frijol de lima a los 45 días de la emergencia de la planta.

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