

(様式 14)

2018 年 08 月 21 日

学位論文の内容の要約

氏 名	ARTIGAS RAMIREZ MARIA DANIELA
学位の種類	博士 (農)
学府又は研究科・専攻	大学院連合農学研究科 生物生産科学 専攻
指導を受けた大学	東京農工大学
学位論文題目	Characterization of nitrogen fixing root nodule bacteria associated with leguminous crops in Venezuela

【論文の内容の要約】

Global population and economic growth are the major forces driving increased world food demand, crop production and fertilizer use. The world needs to increase agricultural productivity in a sustainable and environmentally friendly manner. Research and development of biofertilizer technology have a possibility to provide an alternative way to reduce fertilizer input and establish a sustainable agriculture. Venezuela is considered to have a great biological diversity. However, there is not enough information on biodiversity. Furthermore, in case of the genetic diversity of native rhizobia associated with different legumes in Venezuela, there is not enough information on those. Our work is the first exploration to study symbiotic bacteria and to attempt the developed biofertilizers for various leguminous crops in Venezuela. The objectives are summarized as follows:

1. To elucidate of root nodule bacteria associated with leguminous crops in Venezuela.
2. To characterize genetically the symbiotic bacteria isolated from Venezuelan soils.
3. To characterize abilities of legume symbionts to grow under abiotic stress.
4. To evaluate plant growth promoting traits and symbiotic performance of the isolates, in order to develop candidates of biofertilizers for commonly cultivated and introduced legume in Venezuela such as soybean, cowpea and common beans.

As a result, indigenous rhizobia were collected from 14 different topographical regions in Venezuela. In total 917 isolates were collected from field collection and inoculation test with different 6 legume cultivars and Venezuelan soils. These isolates (917) were characterized for tolerant under stress conditions such as high temperature, acidic and alkaline pH and high concentration of NaCl and Al. Furthermore, 153 rhizobial strains were genetically characterized and the taxonomic position of each isolate was determined by the full nucleotide sequence of 16S rRNA, *nifH*, and *nodD* genes. Characterization of indigenous rhizobia associated with soybean, *Vigna*, and *Phaseolus* crops in Venezuela was as follows: For soybean, a total of 44 indigenous isolates were collected for

phylogenetic analysis. Regarding trap hosts, there is no isolation using *G. max* cultivar 'Enrei' at soils of Guárico, Mérida, and Falcón. Based on 16S rRNA gene sequences, 45% of the isolates were categorized into α -proteobacteria: *Rhizobium* (27%) and *Bradyrhizobium* (18%). Surprisingly, remaining 55% of rhizobial isolates were categorized into β -proteobacteria: *Burkholderia* (55%), this is the first finding of soybean root-nodulating bacteria belonging to *Burkholderia*.

Rhizobia response and their legume-interaction under Al-stress conditions are still unknown. Therefore, we tested responses of rhizobia to soybean under different Al-concentrations. Rhizobial cell numbers were decreased with increase of Al concentrations. Regarding Al-tolerance, intensities of induce of Al-tolerance by soybean rhizobia varied on the rhizobia type and their origins. Interestingly, the secretion of citric acid had a positive correlation with the growth rate and cell density, suggesting that citric acid might be a positive selection force for Al-tolerance and plant-interaction on rhizobia. This finding is also new. Al-toxicity delayed and interrupted the plant-rhizobia interaction and the effect was more pronounced under acidic conditions. *B. fungorum* VTr35 improved the plant growth considerably under acid-Al stress conditions in combination with all soybean varieties. However, the metabolic pathways of rhizobia are still unknown under Al-stress.

Vigna crops are important crops in worldwide and they cultivated in specific areas in Venezuela. However, there are some cultivation problems under acidic and salinity soils. We characterized the Venezuelan rhizobia associated with *V. unguiculata* and *V. radiata*. The phylogenetic diversity showed that root nodules of *Vigna* plant were developed by *Rhizobium*, *Ensifer*, *Bradyrhizobium*, and *Burkholderia* with several new phylogenetic lineages. Some of *Rhizobium* and *Bradyrhizobium* isolates were tolerance to high salinity, Al-toxicity, and high temperature. Similarly, the isolates inducing higher biomass, nitrogen fixation, and root nodulation were *R. phaseoli* isolated from Andes and Amazonas.

For endemic Venezuelan legume, we focused on the two important cultivars of *Phaseolus vulgaris*. This is widely cultivated in Venezuela. It is major Venezuelan diet and their cultivations have several problems in terms of soil acidity, drought, and high temperatures. The phylogenetic positions of the isolates showed that unlike in other continents, Venezuelan *P. vulgaris* was nodulated by α -rhizobia of *Rhizobium*, *Ensifer*, *Bradyrhizobium*, and *Mesorhizobium* and *Burkholderia* of β -rhizobia. In addition, several new phylogenetic lineages were observed into the genus *Bradyrhizobium*. Furthermore, *Phaseolus* isolates showed higher tolerant to high temperature, acid and alkaline pH, salinity stress and high Al-tolerance, their characters were affected by origins of isolates.

The physiological and genetic characterizations of some PGPR were analyzed. In genetic characterization, the major part of Venezuelan isolates tested exhibited nitrogen fixation. Interestingly, 69% of Venezuelan isolates, were classified as *Firmicutes*, mainly *Bacillus* species. One strain developed an effective root nodule showing active nitrogen fixation for soybean Regarding this isolates, we need further characterization to confirm and to validate this root-nodulating bacteria.