DIVERSITY IN THE BEAN NODULATING RHIZOBIAL POPULATION OF NORTH WEST OF ARGENTINA

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1. Introduction

The southern Andes in Argentina is considered to be part of the South American centre of bean domestication. The other centre of origin took place in Mesoamerica. In the NWA region it is possible to find the wild bean *Phaseolus vulgaris* var. *aborigineus* , the ancestors of bean cultivated bean varieties, growing in virgin lands, therefore it is an interesting ecosystem to assess whether the wild bean variety had developed specificity that ended in some particular host-rhizobium association. Another interesting feature of NWA is that the Argentinian bean production resulting of 245,000 Ha of cultivated beans is generated in this region.

It had been demonstrated that *P. vulgaris* is a permissive host, and that several species have been distinguished and new species have been recently described for strains recovered from nodules collected at different sites from all over the world. The majoritiy of isolates from nitrogen fixing nodules of *P. vulgaris* in Mesoamerica are *R.etli,* whereas *R.tropici* have also been isolated from diverse South American regions such as Brazil, Colombia and also in France and Kenyan acid soils. Two additional species *R.gallicum* and *R.giardinii* have been described.

It seems important to gain more information on the occurrence of *Rhizobium* species that nodulate beans in different locations of the NWA. We examined a collection of rhizobial isolates from wild beans growing in virgin lands, and rhizobia that were retrieved from soil in laboratory using common beans and leucaena as the trapping hosts, by using analysis of chormosomal DNA markers such as RFLP-16S rDNA and rep-fingerprinting, and of symbiotic plasmid markers such as RFLP of *nod*C and *nif* H.

2. Experimental procedures

Nodules were surface sterilized with ethanol and hydrogen peroxide, and isolated axenically on YEM-Red Congo medium. Soil isolates were recovered from nodules of plants of common beans or leucaena, that were grown in the laboratory, after inoculation with soil suspension (Aguilar et al., 1998). DNA template for PCR was prepared by using resine chelex-100 in a rapid method described by Alippi and Aguilar (1998). Species assignment of isolates was done by RFLP analysis of PCR - generated 16S rDNA according to the procedure described by Laguerre et al. (1994). The procedure described by de Bruijn was used to generate rep-fingerprinting by using primers REP or ERIC, and dendrograms were obtained by using the computer-assisted system of analysis GelCompar (Applied Maths, Kortrij, Belgium). Variability of symbiotic genes was examined by RFLP analysis of PCR amplified *nod*C and *nif*H, respectively.

3. Results and Discussion

The isolates were obtained from bean collected at different altitudes in a region extended between coordinates 22° 15′ and 27° 21′ latitude S and 64°.40' and 66° 20′ longitude W. Two hundred and eleven isolates obtained from wild beans and 120 isolates obtained from the trapping hosts common beans and leucaena, respectively, were examined. It was found that 64% of isolates of wild beans had a restriction pattern of 16S rDNA identical to *R.etli* while the rest had a pattern identical to

F.O. Pedrosa et al. (eds.), Nitrogen Fixation: From Molecules to Crop Productivity, 187–188.

R.leguminosarum bv. *phaseoli.* Therefore, the wild bean-nodulating rhizobia were found to belong to the type I bean rhizobia but the clear predominancy of the *R.etli* 16S rRNA allele confirms our previous finding obtained with a limited number of isolates (Aguilar et al., 1998). Interestingly, several isolates, from different sites of NWA, had the *R.leguminosarum* 16S rRNA allele. Intraspecies diversity was demonstrated by DNA fingerprinting and RFLP of the 16S-23S rDNA intergenic region. The molecular characterization of the symbiotic genes *nif*H and *nod*C revealed different degree of diversity. It was found by RFLP patterns and amplification of the 3'- coding region of *nif*H (Aguilar et al., 1998), to be highly conserved among the *nif*H sequences of *R.etli* and *R.leguminosarum* bv.*phaseoli* strains. However, by using six different restriction enzyme in RFLP analysis of *nodC* gene, it was found four different patterns (Figure 1). Three of them were shared by both species, whereas one pattern was found only in *R.leguminosarum* bv.*phaseoli.* Two RFLP-*nodC* patterns were identical to the patterns found in the lineages represented by the *R. etli* strains CFN42 and Viking, respectively. Our results demonstrate a limited diversity at the level of species, however fingerprinting and analysis of *nodC* revealed a great intraspecies diversity. The isolates from common beans collected in cultivated areas were found to have the *R.etli* 16S rDNA allele. These results are in agreement with the size of the population type I of bean nodulating rhizobia (e.g. 10^5-10^6 rhizobia g^{-1} of soil). Two rhizobial populations were found associated with the perennial wild bean *Phaseolus augusti,* which shares the habitat with *P.vulgaris* var.aborigineus.The two 16S rRNA alleles were found similar to *B.ekanii* and *B.japonicum* USDA59, respectively.

4. References

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5. Acknowledgements

This work was financed by European Union-INCO (ERB351PL972865) and Secyt-CONICET, Argentina (PID No.331 BID 802 OC/AR). We are grateful to N.Amarger for helpful suggestions.

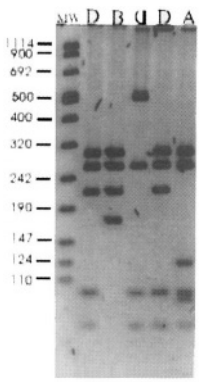

Figure 1. RFLP analysis of *nodC* of rhizobia isolates. The pattern was obtained by using *Hinf*I.