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Evaluation of genetic diversity of bradyrhizobia strains nodulating soybean [*Glycine max* (L.) Merrill] isolated from South Brazilian fields

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ABSTRACT

The massive inoculation of Brazilian soils with few bradyrhizobia strains recommended for soybean has resulted in an established population in most soils cropped with this legume. Besides, several environmental conditions are limiting factors to the growth and activity of rhizobia in soil. These features can shape soil and plant-associated habitats, modifying the composition and activities of their microbial communities. In this work, five bacterial populations from distinct regions of Rio Grande do Sul State were analyzed by rep-PCR and AFLP methodologies. A high level of genetic diversity within populations was observed. The Shannon index was estimated considering a level of 70% of similarity in the profiles and, varied from 3.95 to 6.17 in the different areas. Using the principal coordinate analysis as statistical approach to correlate the bacterial diversity to the soil parameters, it was found that pH, clay and organic matter contents were the major soil factors affecting diversity. Soil pH was the main characteristic that affected bradyrhizobial diversity, whereas clay and organic matter contents had less influence in bacterial diversity. The present study emphasizes that there is a high level of genetic diversity in bradyrhizobia populations that nodulate soybean in Southern Brazilians fields. This information could be useful in the formulation of new inoculants containing strains better adapted to the local environmental conditions, resulting in the improvement of the cropping systems into which these inoculants can be most profitably applied, increasing significantly the productivity of soybean in Brazilian fields.

1. Introduction

Soybean (*Glycine max* L. Merrill), a summer annual herb native from China, is widely cultivated in the South and North Americas. It can establish effective nitrogen fixing symbiosis with species of fast-growing rhizobia (Chen et al., 2000; Hungria et al., 2001, 2006) as well as with species of slow-growing bradyrhizobia, like *Bradyrhizobium japonicum* (Jordan,

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1982), *B. elkanii* (Kuykendall et al., 1992), and *B. liaoningense* (Xu et al., 1995). In Brazil, however, only *B. japonicum* and *B. elkanii* have been used as commercial inoculants to increase soybean yields. As Brazilian soils lack indigenous soybean bradyrhizobia (Freire, 1977; Peres and Vidor, 1980; Ferreira and Hungria, 2002; Alberton et al., 2006), its entire naturalized bradyrhizobia population nodulating soybean possibly came with seeds and inoculants from United States.

The massive inoculation of Brazilian soils with few bradyrhizobia strains recommended for soybean has resulted in an established population in most soils cropped with this legume (Ferreira and Hungria, 2002). These populations show morphological, biochemical, physiological, genetic and symbiotic variability related to adaptation processes and genetic transfer (Paffetti et al., 1996; Santos et al., 1999; Ferreira et al., 2000; Hungria and Vargas, 2000; Ferreira and Hungria, 2002; Galli-Terasawa et al., 2003). Moreover, some studies on bradyrhizobia species isolated from soybean (Abaidoo et al., 2000; Chen et al., 2000), *Lupinus* spp. (Barrera et al., 1997), *Acacia albida* (Dupuy and Dreyfus, 1992), *Aeschynomene* spp. (Wong et al., 1994), and other legumes (Parker and Lunk, 2000; Willems et al., 2003; Wolde-meskel et al., 2004) indicated that the genus *Bradyrhizobium* represents a huge heterogeneous population.

Due to the ecological and economic importance, the bradyrhizobia species and their diversity have been extensively investigated in the last years (Liu et al., 2005). The diversity and the size of indigenous population in soil can vary with the presence of the host legume (Parker, 1999; Andrade et al., 2002) and the history of the land use pattern at the sampling site (Sharma et al., 2005). Several environmental conditions, like extremes of pH, are also limiting factors to the growth and activity of rhizobia in soil (Brockwell et al., 1991; Kahindi et al., 1997; Zahran, 1999). These features can shape soil and plant-associated habitats, modifying the composition and activities of their microbial communities (Paffetti et al., 1996; Bever et al., 1997; Wieland et al., 2001).

Since rhizobia are taxonomically very diverse (Wolde-meskel et al., 2004), efficient strain classification methods are required to identify genotypes displaying, for example, superior nitrogen-fixation capacity (Sikora et al., 2002). Molecular techniques have helped to develop easy and quick methods to microbial characterization including studies discriminating genera, species and even strains (Schneider and de Bruijn, 1996; Botha et al., 2004). The polymerase chain reaction (PCR) and the use of primers corresponding to consensus repetitive sequences dispersed in the eubacteria genome, known as enterobacterial repetitive intergenic consensus (ERIC) and enterobacterial repetitive sequences (BOX) can create highly characteristic patterns when separated in agarose gels (Selenska-Pobell, 1995), providing good discrimination on strain level (Olive and Bean, 1999; Gomez-de-Leon et al., 2000; Saldaña et al., 2003; Wang et al., 2006). ERIC sequences are highly conserved among rhizobia genomes and they were used to distinguish and classify different rhizobia strains in population studies (de Bruijn, 1992; Madrzak et al., 1995; Selenska-Pobell, 1995; Selenska-Pobell et al., 1996; Laguerre et al., 1997; Vinuesa et al., 1998; Chen et al., 2000; Mostasso et al., 2002) and to evaluate the environmental impact in defined populations (Labes et al., 1996).

Quantifying the effects of the factors that best explain the variation of abundance and diversity of communities and populations is a central goal in ecology (Tuomisto et al., 2003). Considering that only four bradyrhizobia reference strains have been widely used in most soybean fields in Brazil, especially in the State of Rio Grande do Sul (RS), the objectives of this work were (1) to characterize and compare the bradyrhizobia nodulating soybean populations from five distinct regions of RS under frequent inoculation; (2) to determine the genetic diversity of the bradyrhizobia populations using rep-PCR and AFLP methodologies; (3) to assess the major soil environmental factors that can affect the local diversity of the bradyrhizobia populations.

2. Materials and methods

2.1. Collection sites and soil samples

Bradyrhizobia nodules were collected from five different regions of RS, Brazil: (1) Ibirubá [28°32′52″S, 53°10′16″W], (2) Cachoeira do Sul [30°02′21″S, 52°53′38″W], (3) Santa Rosa [27°52′15″S, 54°34′50″W], (4) Vacaria [28°30′44″S, 50°56′02″W] and (5) Dom Pedrito [30°58′58″S, 54°40′23″W]. These areas have been used as commercial fields for at least 10 years and have been inoculated with commercial inoculants every 2 years, following standard soil management practices. Ten sub samples of soil (0–15 cm layer) of each field were taken and bulked to obtain a representative soil sample. Sampled soils were analyzed and results are shown in Table 1.

2.2. Bacterial isolates and reference strains

About 100 fresh root nodules from plants growing in fields during the summer (January) were collected from each

Table 1 – Abiotic characteristic of the soils of the sampled sites

Sampled site	pH H_2O	Clay (%)	M.O. (%)	SMP-pH	P (mg dc^{-3})	K (mg dc^{-3})	Fe (g dc^{-3})	Al + H ($cmol_c$ dc^{-3})	Al exc ($cmol_c$ dc^{-3})	Ca exc ($cmol_c$ dc^{-3})	Mg exc ($cmol_c$ dc^{-3})
Ibirubá	6.7	45	4.1	6.8	6.4	227	6.2	1.6	6.3	3.8	6.1
Cachoeira do Sul	6.2	13	2.4	6.8	8	67	1.3	1.7	0	4.1	1
Santa Rosa	6.1	54	4.4	6.2	28	252	1.4	3.5	0	4.5	2.5
Vacaria	5.8	56	6.6	6.2	2.1	261	2.3	3.5	0	6.2	5.1
Dom Pedrito	5.4	24	1.2	5.2	6.8	121	1.8	4.1	0	1.2	4.7

Abbreviations: M.O., organic matter; SMP, potential soil acidity; exc, exchangeable.