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Detection of *Bradyrhizobium* spp. and *B. japonicum* in Thailand by primer-based technology and direct DNA extraction

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Abstract

Total chromosomal DNAs from 20 *Bradyrhizobium* spp. strains (10 strains isolated from *Vigna radiata* and 10 from *Arachis hypogaea*) and 18 *B. japonicum* strains isolated from *Glycine max* were extracted. These DNAs served as templates for REP, ERIC and RAPD primers in PCR analyses. The patterns of the resulting PCR products were analyzed and highly specific for each strain, especially when grouped together with their antibiotic-resistance profiles. A method for extracting DNA directly from soil was developed. Recovery was approximately 30 μg DNA g^{-1} soil and the procedure yielded DNA suitable for PCR amplification.

Introduction

Legumes play a major role in sustainable management of arid areas. Rhizobia have been extensively used in agricultural systems for enhancing the ability of legumes to fix atmospheric nitrogen (Elkan, 1992). One of the impediments to greater nitrogen fixation efficiency is the inability of superior rhizobia used as inoculants to form the majority of nodules under field conditions. Competition from indigenous strains usually limits the performance of the inoculant strains (Keyser and Cregan, 1987). It is therefore important to distinguish inoculant strains from other indigenous rhizobia and to identify the strain responsible for nodulation.

Many methods to identify rhizobia exist but recently new approaches to evaluate the diversity of indigeneous rhizobia using the polymerase chain reaction (PCR) have been developed. PCR can be performed rapidly with strain-, species- or genus-specific primers that generate fingerprints characteristic of each strain. DNA primers corresponding to repetitive palindromic (REP), enterobacteria-repetitive intergenic-consensus (ERIC) coupled with PCR can be used to fingerprint the genomes of rhizobia (De Bruijn, 1993). In addition, short primers of arbitrary nucleotide sequences, RAPD (random amplified polymorphic DNA), have been used since it is simple to detect polymorphisms that allow rapid identification and isolation of chromosome-specific DNA fragments (e.g. Judd et al., 1993; Nick and Lindstrom, 1994; Trevors and Elsas, 1989; Versalovic et al., 1991; Williams et al., 1993).

To fingerprint rhizobia using primer-based techniques, DNA was extracted directly from soil since it overcomes limitations imposed by conventional methods such as the need to culture cells (Sayler and Layton, 1990). We also compared conventional techniques such as antibiotic-resistance profiles with the PCR data.

Materials and methods

Bacterial strains

Twenty *Bradyrhizobium* spp. strains isolated from *Vigna radiata* (L.) *R. Wilezek* and *Arachis hypogaea* L. as well as 18 *B. japonicum* strains isolated from *Glycine max* (L.) *Merr.* are listed in Table 1. TY (Tryptone Yeast-extract: Tryptone 5.0 g, Yeast extract 3.0 g, and $CaCl_2$–2 H_2O 0.1 g per deionized water 1 *l*) was used for cultivation (Beringer, 1974).

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