

Identification of *Sinorhizobium (Ensifer) medicae* based on a specific genomic sequence unveiled by M13-PCR fingerprinting

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Summary. A collection of nodule isolates from *Medicago polymorpha* obtained from southern and central Portugal was evaluated by M13-PCR fingerprinting and hierarchical cluster analysis. Several genomic clusters were obtained which, by 16S rRNA gene sequencing of selected representatives, were shown to be associated with particular taxonomic groups of rhizobia and other soil bacteria. The method provided a clear separation between rhizobia and co-isolated non-symbiotic soil contaminants. Ten M13-PCR groups were assigned to *Sinorhizobium (Ensifer) medicae* and included all isolates responsible for the formation of nitrogen-fixing nodules upon re-inoculation of *M. polymorpha* test-plants. In addition, enterobacterial repetitive intergenic consensus (ERIC)-PCR fingerprinting indicated a high genomic heterogeneity within the major M13-PCR clusters of *S. medicae* isolates. Based on nucleotide sequence data of an M13-PCR amplicon of ca. 1500 bp, observed only in *S. medicae* isolates and spanning locus Smed_3707 to Smed_3709 from the pSMED01 plasmid sequence of *S. medicae* WSM419 genome's sequence, a pair of PCR primers was designed and used for direct PCR amplification of a 1399-bp sequence within this fragment. Additional in silico and in vitro experiments, as well as phylogenetic analysis, confirmed the specificity of this primer combination and therefore the reliability of this approach in the prompt identification of *S. medicae* isolates and their distinction from other soil bacteria. [Int Microbiol 2009; 12(4):215-225]

Keywords: *Sinorhizobium (Ensifer) medicae* · *Medicago polymorpha* L. · rhizobia · nitrogen-fixing nodules · M13-PCR fingerprinting

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