Application Note

ERIC-PCR fingerprinting of indigenous *Sinorhizobium meliloti* strains

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The QIAxcel® system was successfully used to identify and analyze DNA fingerprints of *Sinorhizobium* strains. Analysis using the QIAxcel system involved significantly shorter handling and running times compared to conventional methods, providing an effective, reproducible, and time-saving method for determining genetic diversity in bacteria.

Introduction

Upon infection with rhizobial bacteria, nitrogen-fixing nodules are formed in the roots of legumes. It is a common agricultural practice to inoculate leguminous seeds with nitrogen fixing bacteria such as *Sinorhizobium meliloti* to enhance root nodulation and, subsequently, nitrogen uptake of the plant. Individual strains of *S. meliloti*, however, vary in their symbiotic effectiveness (1). Commercially available inoculants often fail to establish nodules when indigenous rhizobial populations are already present (2). The selection of highly competitive strains is essential for effective inoculation (3, 4).

Indigenous *S. meliloti* strains from different field sites in Croatia were analyzed (4). DNA fingerprints of the enterobacterial repetitive intergenic consensus (ERIC) sequences (5) were established to assess the genetic diversity of the isolates, as well as to establish their relationship to natural populations. The results of the study provided information about nodulation and symbiotic efficiency of individual *S. meliloti* strains (4).

The QIAxcel System.

Materials and Methods

Amplification reactions (25 µl) were prepared with 20 mM Tris·HCl (pH 8.4), 50 mM KCl, 2.5 mM $MgCl_2$, 200 mM each dNTP, 2.5 µl primer, 40 ng genomic DNA, and 1.5 units *Taq* DNA polymerase (Life Technologies). Primers used to fingerprint repetitive ERIC sequences, ERIC 1R and ERIC 2, are described in (6).

Samples were analyzed using the QIAxcel system together with the QIAxcel DNA High Resolution Kit and the 0M700 method with additional 120 second separation time. The QX Alignment Marker 15 bp/3 kb, the QX DNA Size Marker FX174/*Hae*III, and bacteriophage lambda DNA digested with *Eco*47I (*Ava*II) (Fermentas) were included in the run.

Results

The QIAxcel capillary electrophoresis system processes samples in batches of 12 and allows analysis of up to 96 samples without manual intervention. Results can be displayed as a gel-like image as well as an electropherogram. PCR-amplified ERIC sequences of individual *S. meliloti* strains were resolved, exhibiting well separated, sharp bands in the range of 100–2000 bp. A representative gel image is shown in Figure 1. Results were compared with those from 6% poly (NAT) gel (Elchrom Scientific AG) analysis (data not shown). The binary call function of the BioCalculator Software was used to determine the presence or absence of specific fragments in the samples. DNA sizes were precisely and reproducibly calculated. The results were used in a later study to prepare a dendrogram displaying the relatedness of the isolated strains.

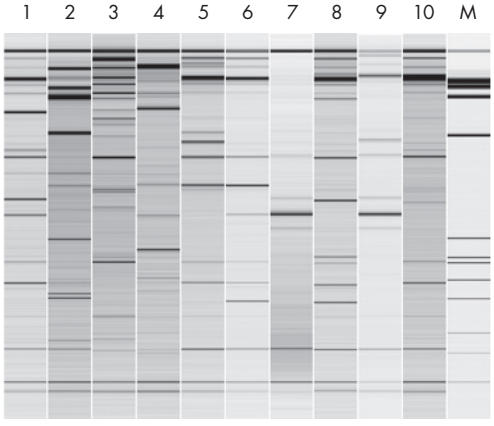

Figure 1. Detection of genetic diversity for *Sinorhizobium meliloti*. PCR amplified ERIC sequences of individual *S. meliloti* strains were analyzed using the QIAxcel DNA High Resolution Kit. **1–9**: Strains isolated from the nodules of alfalfa (*Medicago sativa* L.); **10**: reference strain 2011; **M**: QX DNA Size Marker FX174/*Hae*III.

Conclusions

- ERIC PCR fragments were separated and unambiguously identified using the QIAxcel system and BioCalculator Software. Subsequent comparison of the ERIC PCR patterns led to the precise determination of the genetic relationship of bacterial strains.
- The QIAxcel DNA High Resolution Kit resolves ERIC PCR fragments more effectively than agarose or polyacrylamide gels, providing greater sizing accuracy and improved sensitivity. The results are fully reproducible due to controlled running conditions and automated data acquisition. Since up to 96 samples can be analyzed in a single run, the QIAxcel system yields more information from DNA fingerprints while saving time.
- Since the QIAxcel capillary electrophoresis uses only minute quantities of DNA through electrokinetic injection, the samples are retained for downstream procedures, such as sequencing or cloning.

References

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Ordering Information

Product	Contents	Cat. no.
QIAxcel Advanced System	Capillary electrophoresis device, including computer, and ScreenGel Software; 1-year warranty on parts and labor	9001941
QIAxcel DNA High Resolution Kit (1200)	QIAxcel DNA High Resolution Gel Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929002
QIAxcel DNA Screening Kit (2400)	QIAxcel DNA Screening Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929004
QIAxcel DNA Fast Analysis Kit (3000)	QIAxcel DNA Fast Analysis Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, QX DNA Size Marker 50 bp – 1.5 kb, QX Alignment Marker 15 bp/3 kb, 12-Tube Strips	929008
QIAxcel RNA QC Kit v2.0 (1200)	For 100 runs of 12 samples: QIAxcel RNA Quality Control Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, QX RNA Alignment Marker, QX RNA Size Marker 200–6000 nt, QX RNA Denaturation Buffer, 12-Tube Strips	929104

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