magtivio

Steinbrenner Laborsysteme GmbH
In der Au 17
69257 Wiesenbach
+49 (0) 6223 / 96 73 00
mail@steinbrenner.de
www.steinbrenner.de

MagSi-DNA Soil

Application Note | DNA extraction from soil samples for microbial profiling

Introduction

Soil is a complex environment governed by abiotic parameters such as soil texture, water content, pH and climatic variations. These interacting parameters play a key role in modulating microbial diversity and their indirect effect on plants. Microorganisms are heterogeneously distributed throughout soil and strongly bind with soil particles. They perform several essential functions such as soil formation, toxin removal and nutrient cycling (e.g. carbon, nitrogen, phosphorus) which makes them indispensable to maintain good soil health. By interacting with plant roots, microbes also have a great influence on the development, health and survival of plants in unfavourable conditions. Therefore these beneficial plant-microbe interactions are of particular interest to the agricultural industry.

This application note describes the extraction of microbial DNA from 2 different soil types (black soil and sandy loam) using the MagSi-DNA soil kit. In addition, subsequent investigations of the soil microbial community are performed based on a selection of 4 bacterial species that are commonly isolated from soil environments and microbial profiling of the 16S ribosomal RNA.

Materials and methods

Soil samples were collected in Nuth (black soil) and Lanaken (sandy loam) and stored at 2-8°C until microbial DNA extraction with the MagSi-DNA Soil kit. 250 mg of the various soil samples was added to grinding tubes and mixed with 550 µL Lysis Buffer SL and 20 µL Proteinase K. The samples were lysed by bead beating in the 2010 Geno/Grinder® (SPEX® SamplePrep, 5x 1 min, 1500 rpm) followed by lysis incubation at 70°C for 10 minutes (ThermoMixer C, Eppendorf). After a brief centrifugation step (10 min, 18 000 x g), 300 µL sample lysate was transferred to new 2 mL tubes. 100 µL Precipitation Buffer SL was added and the samples were incubated at 2-8°C for 10 min. After centrifugation (10 min, 18 000 x g), 300 µL sample lysate was used as input for DNA extraction on the PurePrep 96 Nucleic Acid Purification System with a final elution volume of 150 µL.

Soil DNA quantity and purity were assessed by UV-VIS spectroscopy with the NanoDrop™ One (ThermoFisher Scientific™) according to manufacturer instructions. DNA integrity was evaluated by automated gel electrophoresis
on the 4150 TapeStation (Agilent) with a Genomic DNA ScreenTape. DNA quality and absence of inhibitors were evaluated by quantitative real-time PCR with universal 16S ribosomal RNA (rRNA) primers (AriaMx Real-Time PCR system, Agilent). After pre-amplification, a second PCR was performed with species-specific primers targeting common soil bacteria *Xanthobacter autotrophicus*, *Paenibacillus sabinae*, *Pseudomonas protegens* and *Arthrobacter chlorophenolicus* (according to Kleyer et al. (2017)). The reactions consisted of 2 µL template DNA in a total reaction volume of 20 µL (primaQUANT CYBR qPCR Master Mix, Steinbrenner Laborsysteme).
Eurofins Genomics amplified and sequenced the V3-V4 hypervariable regions of the 16S rRNA gene on Illumina MiSeq with the 2 x 300 bp paired-end read module. Raw sequencing data was processed using Cutadapt software to remove adapter sequences, primers, poly-A tails and other unwanted sequences from the high-throughput sequencing reads. Primers removed paired-end reads were merged with a minimum overlap size of 10 bp to reduce false positive merges using the software FLASH. Chimeric sequences were removed and the remaining reads were partitioned into Operational Taxonomic Units. The taxonomic assignment was completed, generating a list of the most specific taxonomic units, their taxonomy level and fraction.

Results and discussion

DNA yield

Soil DNA yield is presented in Figure 1. The nucleic acid yield depends on the soil type, showing the highest yield for sandy loam (3.18 µg). The lowest yield was obtained for the dark soil collected in Nuth. The content of organic matter, clay, silt, water and pH are the main factors affecting the quality and quantity of the obtained DNA. This explains the observed variation in DNA yield between the 2 investigated soil types.

magtivio B.V.

Daelderweg 9
6361 HK Nuth | The Netherlands

Tel.: +31 (0)45 208 4810
Fax: +31 (0)45 208 4817
www.magtivio.com
info@magtivio.com

magtivio

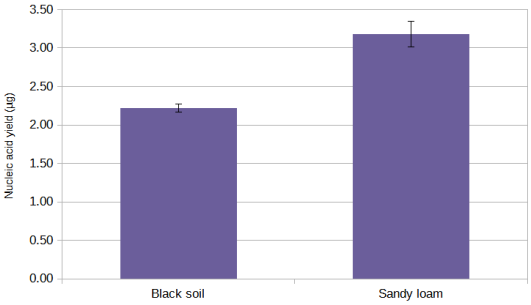

Figure 1. *DNA yield obtained from different soil samples using UV-VIS spectroscopy (NanoDrop™ One). The DNA recovery ranges from 2 to 3.5 µg depending on the soil type. DNA was eluted in 150 µL. The data are presented as mean (n= 5, ±1 SD).*

Purity

UV-VIS spectroscopy provides data for evaluating the DNA purity of the soil samples (Table 1). The A260/A280 absorbance ratio is ≥1.5 for both soil types. The A260/A230 absorbance ratio shows more variation with values ranging from 1.4 to 1.8.

Table 1. Purity ratios of soil DNA by UV-VIS using the NanoDrop™ One.

Soil sample	A260/A280	A260/A230
Black soil	1.70	1.43
Sandy loam	1.67	1.79

Data are presented as mean (n=5)

DNA integrity

Extracted soil DNA was visualized via automated gel electrophoresis on a genomic DNA screentape (Figure 2). DNA integrity numbers ranged from 5.7 to 7.0, indicating only minor DNA degradation related to sample collection, storage and processing. The strong gel bands represent a large fraction of high molecular weight DNA. Having high molecular weight DNA is beneficial for PCR as it decreases the formation of chimeras during PCR of samples with mixed templates, such as environmental samples.

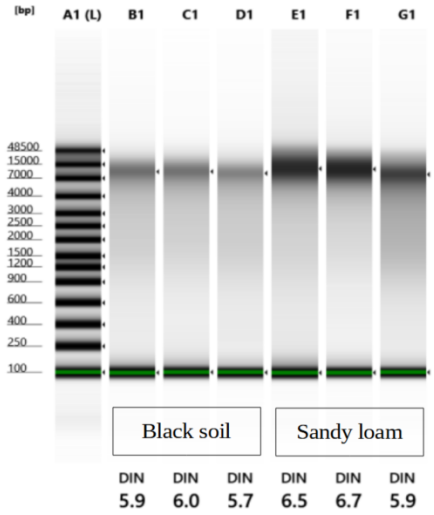

Figure 2. *Automated gel electrophoresis image with DNA integrity numbers (DIN, 1-10) for DNA extracted from soil samples. Trace A1: Ladder, B1-D1: black soil, E1-G1: sandy loam.*

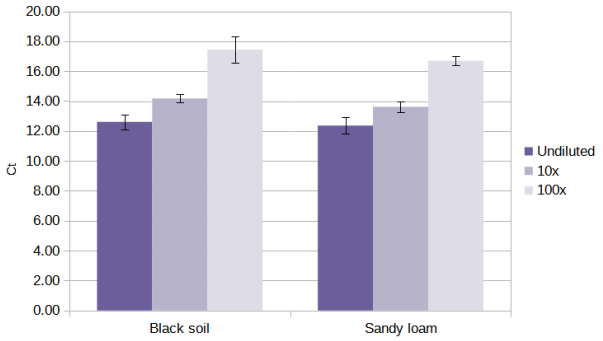

Figure 3. *Inhibitor-sensitive 16S rRNA PCR on soil eluates after extraction with the MagSi-DNA soil kit. The soils show no PCR inhibition. The data are presented as mean (n= 5, ±1 SD).*

After pre-amplification, a second PCR to detect 4 common soil bacteria was performed (Figure 4). In general, *X. autotrophicus* and *P. protegens* are the most prevalent bacteria in the investigated soil types. *X. autotrophicus* are important plant growth promoting bacteria while *P. protegens* are known for their plant-protecting characteristics. *P. sabinae* and *A. chlorophenolicus*, two common species that support plant growth, are present to a lesser extent. The ability to detect specific bacterial species and their relative abundance proves that the MagSi-DNA soil kit generates high quality DNA that can be used to study plant-microbe interactions.

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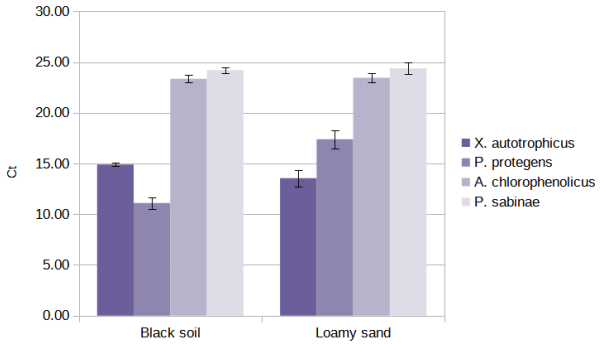

Figure 4. *Species-specific PCR after pre-amplification of soil samples. Note the abundance of X. autotrophicus and P. protegens in both soil types. The data are presented as mean (n= 5, ±1 SD).*

Microbial community composition

16S rRNA sequencing was used to identify the microbial profile of the soil samples. The sequencing revealed that Actinobacteria (30-50%) and Proteobacteria (35-50%) were the most prevalent bacteria in the investigated soil samples (Figure 5). Actinobacteria are known as one of the most abundant phyla in soil bacteria. They are involved in biogeochemical cycling of carbon, nitrogen, phosphorus and other elements. A major fraction of animal and plant residue decomposition is performed by their hydrolytic enzymes. Also Proteobacteria are one of the dominant phyla in soil samples actively contributing to the cycling of various nutrient elements. The relative abundance of bacteria at phylum level confirms the quality of extracted DNA and efficient removal of inhibitors.

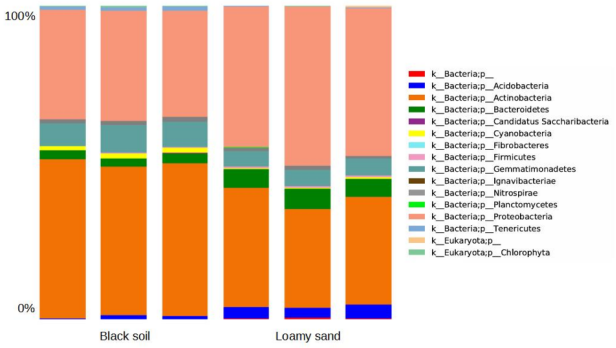

Figure 5. *Microbial community profiles from soil samples obtained by sequencing of 16S rRNA genes (V3-4 region). The identified microbial taxonomic units are summarized at phylum level (n=3).*

Conclusion

MagSi-DNA Soil can be successfully used for DNA extraction from different soil types. The majority of inhibitors is efficiently removed and the obtained DNA with high integrity can be used directly in downstream molecular analyses to investigate soil bacterial community compositions.

Literature

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Ordering information

Art. No.	Description	Amount
MDKT00280096	MagSi-DNA Soil	96 preps
AS00001	PurePrep 96 Nucleic Acid Purification System	1 unit
MDPL00200050	PurePrep 96 Deepwell Plate	50 pcs
MDPL00190060	PurePrep 96 Elution Plate	60 pcs
MDPL00210060	PurePrep TipComb	60 pcs

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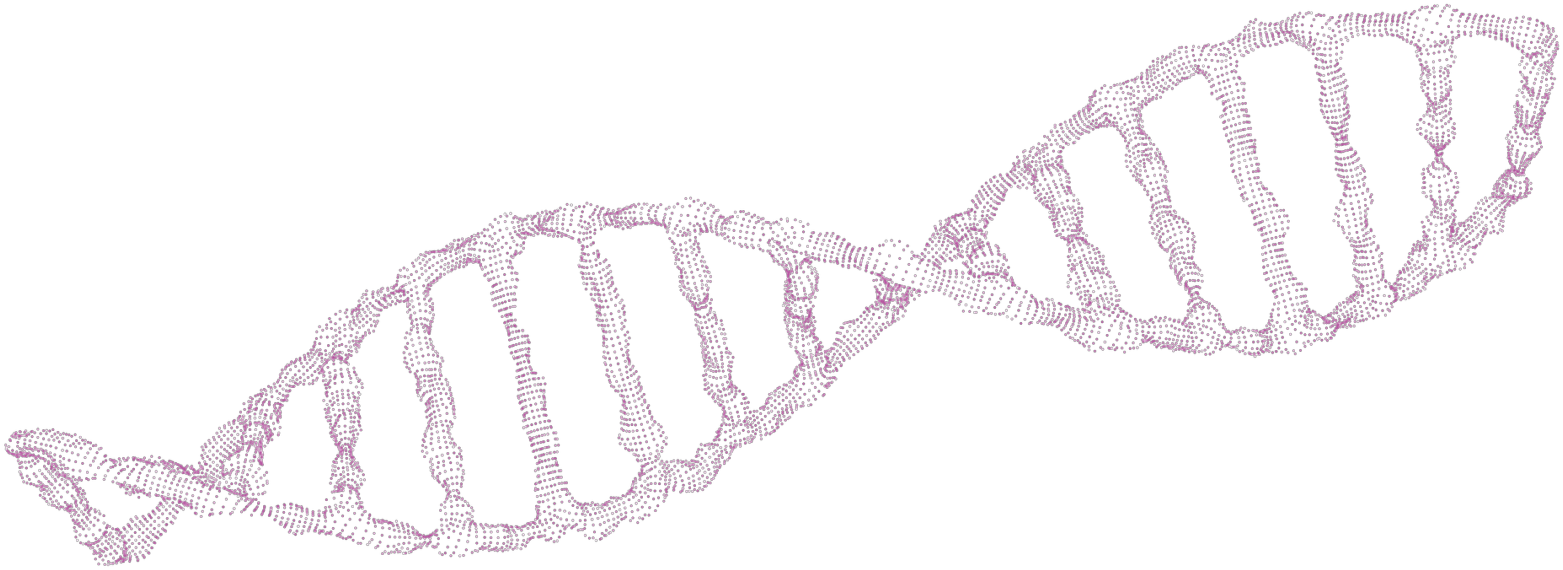

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