Institute of Soil Science and Plant Cultivation

Identification of dominant bacteria in different types of soil using PCR-DGGE molecular standards

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INTRODUCTION

The development of **molecular standards** in this project will allow for a better understanding of the **dominant species** involved in the life of the soil. In addition, DGGE analysis using molecular standards can be a **routine method** for rapid monitoring of changes occurring under different factors such as agrotechnical treatments or changing atmospheric conditions. This will be an extremely valuable method for quick results.

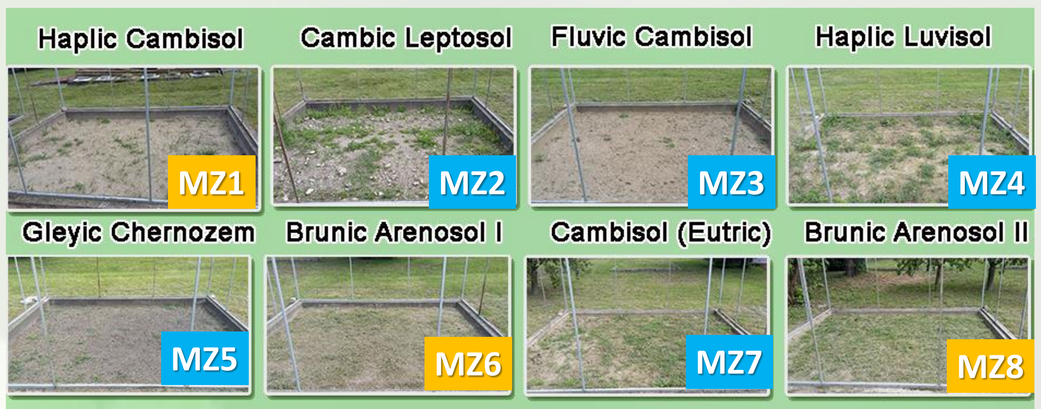
Standards were prepared by cutting out the most distinctive and clear bands from DGGE gel. Afterwards the bands were **cloned** into chemically competend *E.coli* cells. Transformed bacteria colonies were screened by a colony-PCR method and PCR-DGGE confirmation. Bacteria containing expected DNA fragments were stored in -80°C as a PCR-DGGE **molecular standards bank**.

At this stage of the study, subcloned sequences are being collected and then will be sequenced. In a parallel experiment NGS (MiseQ, Illumina) sequencing of entire soil populations was carried out. The results of this experiment, combined with NGS results, will allow the question whether PCR-DGGE is an appropriate method for rapid screening of predominant bacterial species.

This poster presents partial results and methodological aspects of the research that is still in progress.

EXPERIMENT DESIGN - MICROPLOTS

Founded in 1881, eight microplots collecting different types of soil

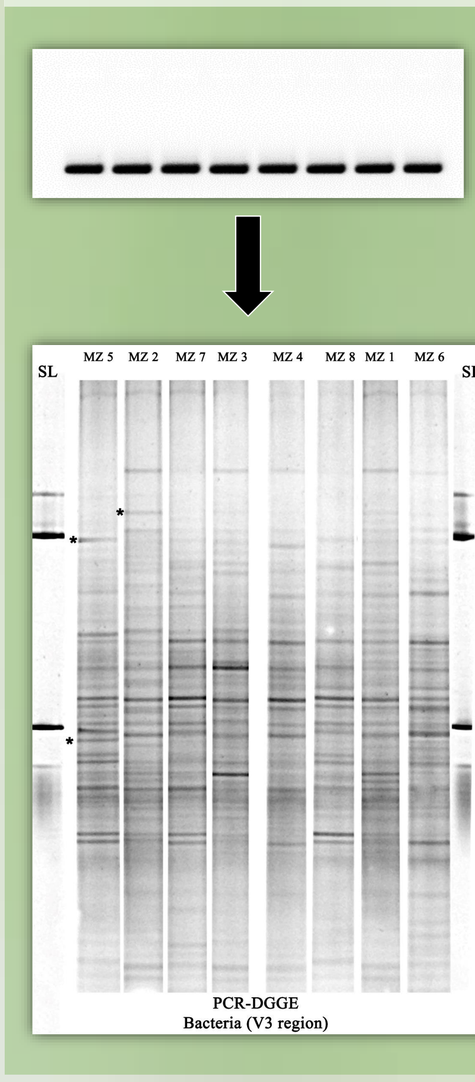


MZ(1-8) abbreviations are used in subsequent figures.

Poor quality, strongly acidic soils: Haplic Cambisol, Brunic Arenosol (I and II)

Good quality soils: Gleyic Chernozem, Cambisol (Eutric), Cambic Leptosol, Fluvic Cambisol, Haplic Luvisol

Standard PCR vs PCR-DGGE



Denaturing Gradient Gel Electrophoresis (DGGE) allows the separation of fragments of the same size.

Different bands position on the gel results from **different melting temperatures** of DNA molecules. Higher GC content results in higher melting temperature, and thus further migration. This method allows to distinguish sequences **differing even by several nucleotides.**

The intensity of individual bands results from different concentrations of PCR products, and thus depends on the initial amount of DNA. The species of bacteria that predominate in a given environment are characterized by more intense bands.

However, care should be taken in quantitative interpretations, as different quantities of PCR products may also depend on the number of 16S rDNA copies in a cell that is variable between individuals or species.

PCR-DGGE Similarity matrix vs NGS Clustering

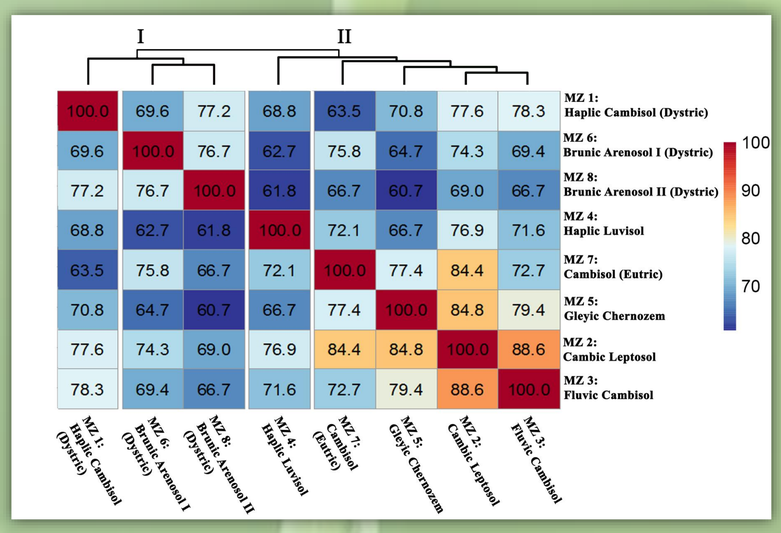
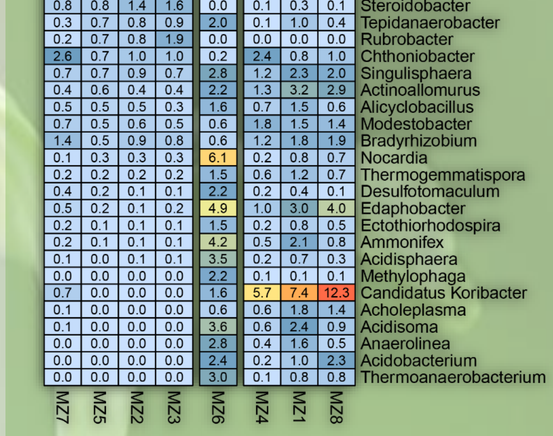
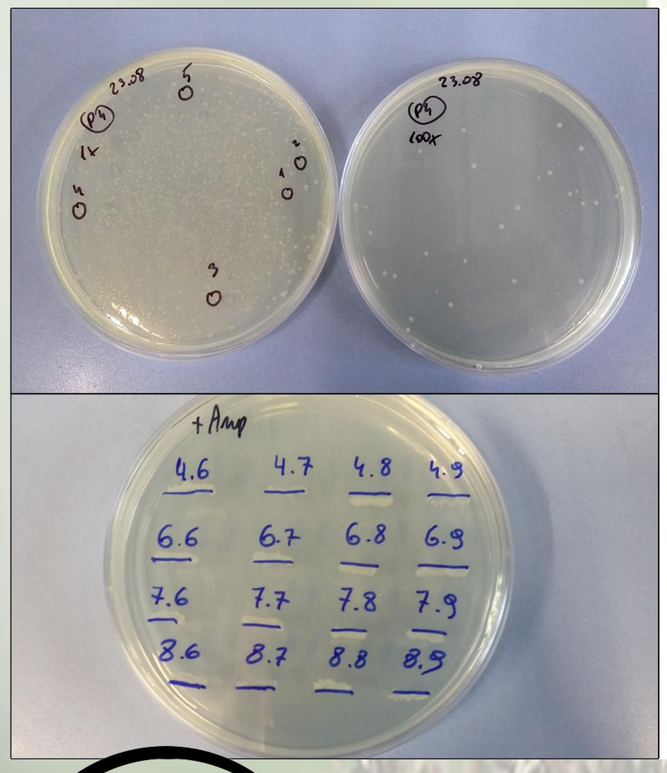
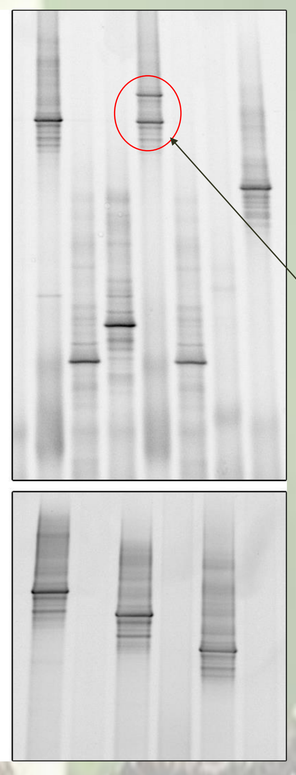


Image after PCR-DGGE analysis was analyzed in QuantityOne (BioRad) software. Similarity matrix was generated based on bands **positions pattern**, as well as their **intensity**.

The most similar soil types (similar pH, quality, organic carbon content etc.) were clustered into the same groups. **Acidic soils** (MZ 1,6,8) were clustered in the group I, while II group clustered good quality soils (MZ 2,3,4,7).







Cloning of DGGE bands

Sequencing of the single band, excised from the gel, requires **reamplification** and subsequent **cloning** into a **plasmid vector**. Cloning ensures that exactly one PCR product is obtained.

A single band from one bacterial species is expected, but it is often issue (as a result of different possible structures of the same DNA), that one DNA molecule is present at **several levels** on the DGGE gel. This sometimes may lead to misinterpretations and **overestimation** of the overall number of species present in a sample.

As a result of the Next Generation Sequencing data analysis (pheatmap package, Rstudio) similar clustering of different soil types was obtained. NGS showed more accuracy and sensitivity, for example MZ6 (the most acidic soil, pH 4.0) was extracted into separate subgroup), MZ4 (slightly acidic, but good quality) share more common bacteria composition with acidic soils).

Summary

The most distinctive and dominant bands from PCR-DGGE was succesfully reamplified and cloned into *E.coli* cells, serving as a molecular standards for further experiments. Next generation sequencing of eight soil types microbiota was carried out, and sequencing of DGGE bands is forthcoming.

This method will be used to monitor changes in biodiversity in commercial soils, as well as to measure the activity of microorganisms under varying conditions of the usage.

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