

# Quantitative PCR as a tool for analysis of diversity in soil microbiome

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## Abstract

Majority of terrestrial soils are used primarily for agricultural production. The main factor of soil fertility is mediated by soil microorganisms and invertebrates. Soil microorganisms plays crucial role in ecosystems nutrient cycling and therefore any change in their diversity could have serious consequences on soil fertility and agricultural yields. Silver nanoparticles as novel potentially nontoxic compounds are nowadays used in many daily use products and also as pesticides. But based on their antimicrobial activity, they can adversely influence structure of soil microbiome. Our work is focused on qPCR analysis of soil microbiome diversity with and without silver nanoparticles treatment. Phylogenetic analysis revealed concentration dependent shifts in microbial diversity, usually in the favor of one phylogenetic group of bacteria or fungi and analysis of melting curves showed changes inside individual phylogenetic groups.

**Keywords:** nanoparticles, soil microbiome, phylogenetics

## 1. Introduction

The diversity of the bacterial and fungal communities is extraordinary. Such high level of diversity make quantifying and characterizing of soil microbial communities difficult (Torsvik et al., 2002). 16S rRNA and ITS region currently represent the most important targets of study in bacterial and fungal ecology, including the determination of phylogenetic relationships among taxa, the exploration of microbial diversity in the environment and the quantification of the relative abundance of taxa of various ranks (Hugenholtz et al., 1998). For quantification of relative abundances sets of primers restricting sequence specific for different bacterial or fungal phyla were designed (Fierer et al., 2005; Nikolcheva and Bärlocher, 2004). One of the most used techniques for quantification of abundance of microbial phyla is quantitative or real-time PCR. In our study we exploited qPCR for quantification of relative abundances of several bacterial and fungal phyla in soils influenced with silver nanoparticles. Also we analyzed process of melting

curves of individual amplicons. Melting temperature of DNA sequence is more dependent on GC-pairs ratio than on its length, taking higher GC content significantly increased stability of DNA and therefore increase the melting temperature. This property is successfully exploited in methods such PCR-TGGE or PCR-DGGE.

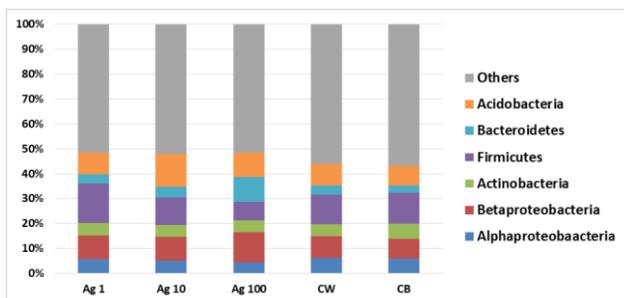
## 2. Materials and Methods

Experimental soil was prepared from sterilized garden substrate and inoculation soil originating from non-agricultural area and treated with distilled water (growth control), citrate buffer (solvent control) or spherical silver nanoparticles with diameter of 20-25 nm. The cultivation was realized on the Institute of Experimental Botany CAS under following conditions: growth for 4 weeks in growth chamber Snijder at 22 °C, 70 % humidity, 10 / 14 h (light / dark), applied concentration of silver nanoparticles was 1; 10; 100 mg/kg of dry soil, watering with distilled water to 65-70 % WHC. The experiment was realized in two parallels, the first one was seeded with *Arabidopsis thaliana* Col-0, second one was unseeded. After the cultivation time has elapsed, plants and soils were harvested. Soil samples intended for qPCR analysis were immediately stored in -80 °C. Total soil DNA was isolated using FAST DNA<sup>TM</sup> SPIN KIT for Soil (MP Biomedicals, FR). qPCR was realized on LightCycler® 480 (Roche, DE). The PCR reaction was performed using LightCycler® 480 SYBR Green I Master (Roche, DE) and taxon-specific primers (Fierer et al., 2005; Nikolcheva and Bärlocher, 2004) according manufacturers instructions.

## 3. Results

### 3.1. Determination of relative abundances of selected bacterial phyla

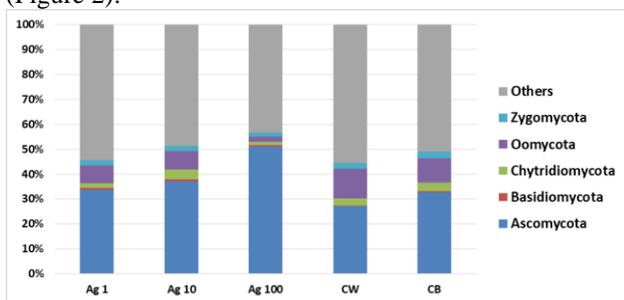
qPCR analysis clearly proved concentration dependent shift in relative abundance of bacterial phyla in the favor of Betaproteobacteria and Bacteroidetes at the expense of other followed phyla (Figure 1).



**Figure 1:** Relative abundances of selected bacterial phyla in seeded soil. Ag 1; 10; 100 - treatment with AgNPs - 1; 10; 100 mg/kg soil; CW - growth control; CB - solvent control.

### 3.2. Determination of relative abundances of selected fungal phyla

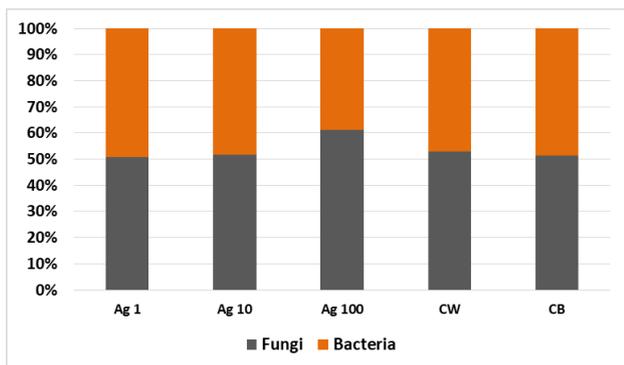
qPCR analysis clearly proved concentration dependent shift in relative abundance of fungal phyla in the favor of Ascomycota at the expense of other followed phyla (Figure 2).



**Figure 2:** Relative abundances of selected fungal phyla in seeded soil. Ag 1; 10; 100 - treatment with AgNPs - 1; 10; 100 mg/kg soil; CW - growth control; CB - solvent control.

### 3.3. Determination of relative abundances of bacteria and fungi

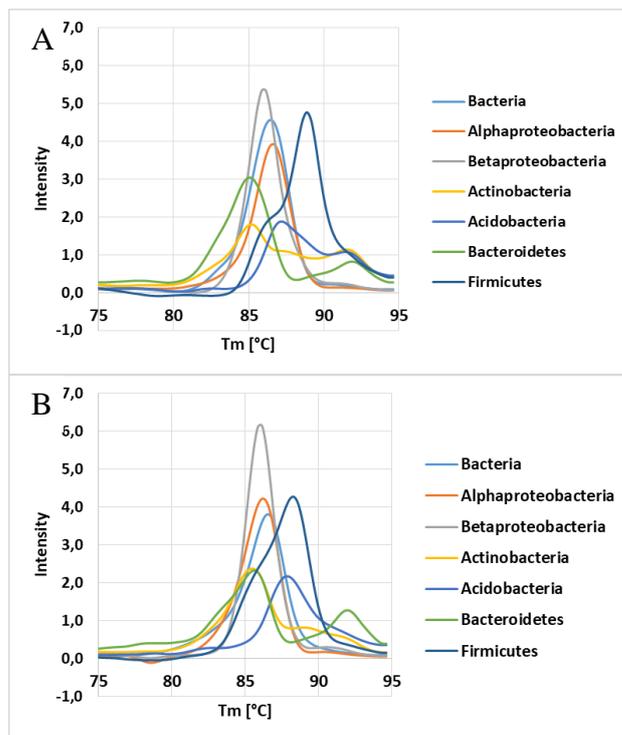
qPCR analysis clearly proved concentration dependent shift in relative abundance of fungi at the expense of bacteria (Figure 3)



**Figure 3:** Relative abundance of bacteria and fungi in seeded soil. Ag 1; 10; 100 - treatment with AgNPs - 1; 10; 100 mg/kg soil; CW - growth control; CB - solvent control.

### 3.4. Analysis of melting curves

Analysis of melting curves clearly showed shift in melting temperatures and therefore in GC content of amplified sequences which indicate changes in diversity of genera and species inside the phylum (Figure 4 A, B).



**Figure 4:** Melting curves of bacteria and bacterial selected bacterial phyla without treatment (A) and treated with AgNPs in concentration equal 100 mg/kg soil (B).

## 4. Conclusions

Using qPCR technique we were able to determine changes in relative abundance in bacterial and fungal phyla in samples without treatment and treated with silver nanoparticles. Analysis of melting curves indicated changes in abundance of genera and species, which will be clarified using PCR-DGGE.

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