Complex approach for analysis of changes in soil microbiome

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Abstract
In the few past decades, genetic approaches for analysis of soil microbiome, e.g pyrosequencing, gained major attention and cultivation techniques were pushed into the background. The combination of several techniques could be useful and cheap approach for analysis of changes in soil microbiome. In our study of effect of silver nanoparticles on soil microbiome we exploited classical microbiological and biochemical methods for assessment of microbial structure. Effect caused by addition of silver nanoparticles was concentration dependent, the highest concentration of silver nanoparticles caused, besides the others, significant decrease in dehydrogenase activity of soil microorganisms. Concentration dependent changes in soil microbiome were also detected, e.g. in total numbers of cultivable microorganisms, cellulolytic or peroxidase. CLPP indicated significant shift in microbial diversity in all levels of substrate utilization.
Keywords: nanoparticles, soil microbiome, toxicity

1. Introduction

Soils are the foundation of all terrestrial ecosystems and are home to a vast diversity of bacteria, archea, fungi, insect, and other invertebrates as well as plants and algae. Soils and their dwellers provide food and nutrients for above and below ground organisms. Soils are critical segment in filtering system of freshwater ecosystems. Consequently, soils are extremely important for humans (Dominati et al., 2010). Soil microorganisms play diverse and often critical roles in ecosystems. The majority of soil microbial activities contribute to the cycling of all major elements and consequently affect the structure and the functions of soil ecosystems.

For thousands of years soils provide food for humankind. With increasing population the need for more food grows. In last century this need resulted in enormous exploitation of fertilizers and pesticides, often based on heavy metals. In the last few decades scientists proved toxic effect of heavy metals on almost every life form. These findings started the era of ecological approaches in agriculture and application of novel potentially nontoxic compounds, like zero-valent metal nanoparticles. Silver was used as antimicrobial agent and for covering of severe wounds and burns in history. This property, extra enhanced in nano-form of the metal, made from it one of the most used material in human life. Nano-silver is nowadays used, except of electronics, e.g in cosmetics and textile (Ratte, 1999). Daily use of ware containing nano-silver increase the risk of its release into to environment, where as any other metal it will accumulate into the potentially toxic concentrations (Buzea et al., 2007).
The aim of this work was to determine the effect of silver nanoparticles on soil microbiome with exploitation of cultivation and non-expensive techniques.

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 and analyzed within one month. The realized analyzes concerned soil physico-chemical properties (soil dry weight, pH, cation exchange capacity, water holding capacity), composition of organic matter (total organic carbon, total reducing sugars, hexoses and their polymers, pentoses, DNA, amino acids and α-amino-N and proteins) (Badalucco et al., 1992), community level profiling (total number of cultivable microorganisms, antibiotic resistance, CLPP (Weber and Legge, 2010), FAMEs, ergosterol, qPCR), biochemical analysis (antioxidant, dehydrogenase, cellulose, peroxidase, amidase, urease, catalase, amylase, keratinase and protease activities) (Serra-Wittling et al., 1995) and growth promoting activities (phosphate solubilization, indole-3-acetic acid production, ammonia production, hydrogen cyanide production, siderophore production, nitrogen fixation and ACC-deaminase activity).

3. Results

3.1. Composition of organic matter

Analysis of composition of organic matter is useful in determination of quantity of soil microbiome. In our study this analysis did not demonstrate any changes between samples, which could indicated that possible changes are in structure not in amount of soil microbiome.

3.2. Community level profiling

Determination of antimicrobial resistance with a spectrum of antibiotics indicated concentration dependent shift of soil microbiome in different bacterial groups. Determination of ability to utilize ecologically important substrates, i.e CLPP indicated significant shift in microbial diversity in both seeded and unseeded soils in all levels of substrate utilization (Figure 1).

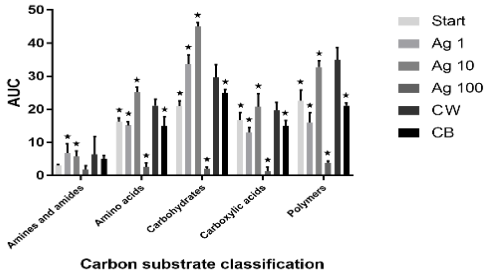

Figure 1. Ability of utilization of different carbon substrate class. Ag 1; 10; 100 - treatment with AgNPs - 1; 10; 100 mg/kg soil; CW - growth control; CB - solvent control; Start - original soil. α=0,05.

3.3. Biochemical analysis

Determination of enzymatic activity indicated the shift of microbial community in the favor of microorganisms capable to utilize polymeric substrates (Figure 2). Determination of peroxidase activity showed higher production of peroxidases in the highest NPs concentration treatment, which indicates generating of oxidative stress Figure 3).

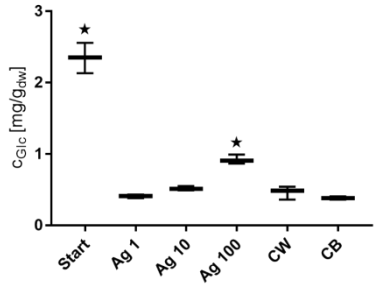

Figure 2. Activity of cellulases expressed as concentration of glucose cleaved from carboxymethylcellulose. Ag 1; 10; 100 - treatment with AgNPs - 1; 10; 100 mg/kg soil; CW - growth control; CB - solvent control; Start - original soil. α=0,05.

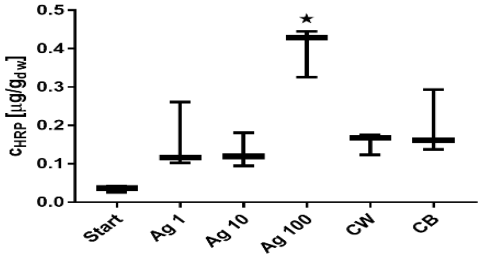

Figure 3. Concentration of peroxidases expressed as horse radish peroxidase. Ag 1; 10; 100 - treatment with AgNPs - 1; 10; 100 mg/kg soil; CW - growth control; CB - solvent control; Start - original soil. α=0,05.

3.4. qPRC

With quantitative PCR were followed changes on phylogenetic level of soil microorganisms. This analysis clearly proved concentration dependent shift in relative abundance of bacteria and fungi. Analysis of abundance of individual bacterial and fungal phyla showed again concentration dependent shift in the favor of Betaproteobacteria and Bacteroidetes and Ascomycota, usually at the expense of other followed phyla.

4. Conclusions

Cultivation techniques indicated changes in soil microbiome on qualitative level. Genetic and analytic approaches clarify these changes group and phylum levels.

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