COMPARATIVE METAGENOMIC ANALYSIS OF THE HYDROLYTIC PROCARYOTIC COMPLEXES OF MODERN AND BURIED CHESTNUT SOILS AND BURIED PERMAFROST SOILS

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BACKGROUND

The properties of paleosols as indicators of biosphere development gain increasing attention of researchers for evaluation of the possible consequences of global changes of environment and climate. Recently the subject of paleomicrobiome long-term sustainability mechanisms and transformation of microorganisms required for survival in subsurface sediments became a subject of great scientific interest. The question about functional activity and structure of microbiological community in subsurface sites remains open. Proven that microbiological community stays “preserved” and keeps original properties from the moment of burial, paleosoils can be considered as natural collections of microorganisms and may have a great biotechnology potential. Previously, the authors revealed the fact that the intensity of response to the introduction of the substrate increased with the deposition depth and age of the soil (Manucharova et al., 2014). But the question about species (or genera in case of prokaryotic complexes), which are responsible for the intensification of microbial biomass multiplication in subsurface sites, remained unclear.

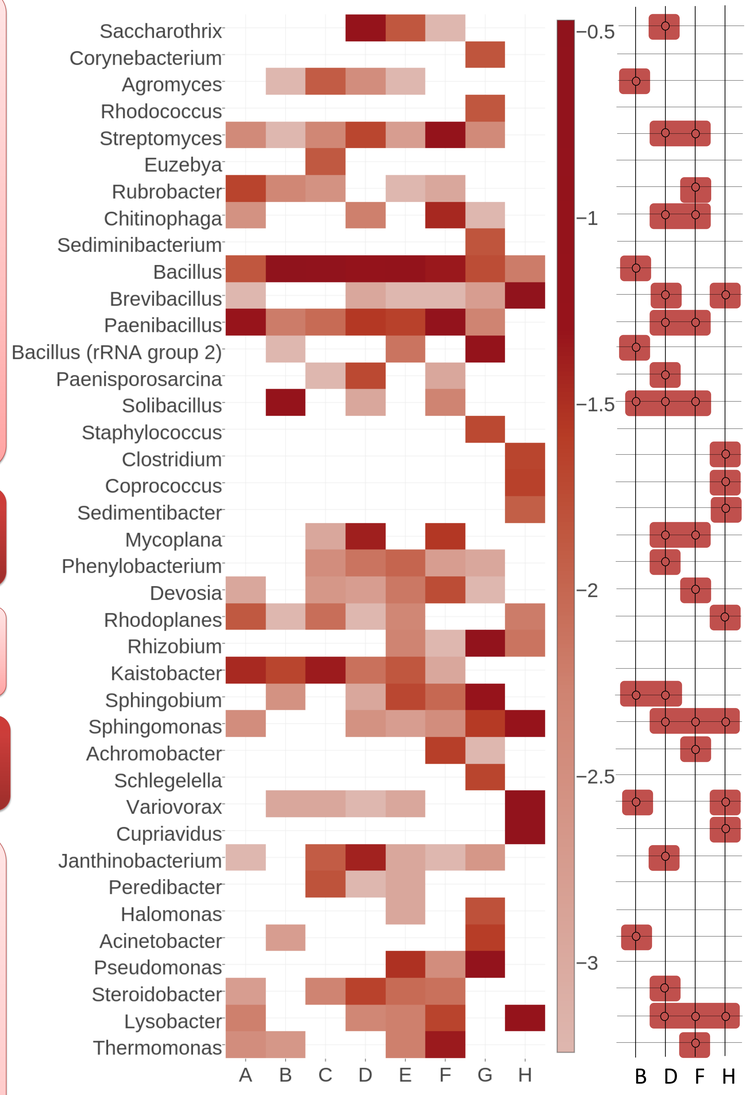
AIM OF THE STUDY

The comparative metagenomic analysis of the hydrolytic procaryotic complexes of modern and buried chestnut soils and buried permafrost soils

MATERIALS & METHODS

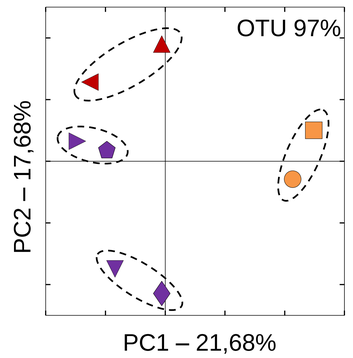
Subjects of the study were the buried subkurgan paleosoils (deposition depth 0.5 and 2.5 m, burial age 3500 and 4500 years respectively), modern chestnut soils and buried permafrost marine terrace sediments (deposition depth 9 m). The structure of the hydrolytic microbial complex was determined by the microcosm method with initiation of microbial succession by humidification and introduction of purified chitin (ICN Biomedicals, Germany) at concentration of 0.2%. Soil humidified with water (1 mL/5 g soil) without a substrate was used as a control. For DNA extraction the PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Inc., USA) and protocol were used. The metagenomic analysis was performed with next generation sequencing (454 sequencing) on the Genome Sequencer FLX (Roche, Switzerland) with GS FLX Titanium series reagents and protocol. PCR-fragments of metagenomic DNA samples were obtained with degenerated primers PRK341F и PRK806R. Analysis of the data was performed in QIIME (Caporaso et al., 2010). OTU picking at the similarity levels 97%, 94%, 91%, 88%, 85%, 81% was performed with use of UCLUST algorithm (Edgar, 2010), all the reads were aligned via PyNAST (Greengenes) (Caporaso et al., 2010; DeSantis et al., 2006). Taxonomy was assigned according to RDP classifier (Wang et al., 2007) and the phylogenetic tree was made with FastTree algorithm (Price et al., 2010).

RESULTS



Dominant bacterial genera and their relative share, illustrated by heatmap (OTUs are defined at the 97% similarity level; genera representing more than 1% of all defined OTUs are considered dominant; figure shows the Lg values). The sheme on the right represents the genera, which share is increasing after introduction of substrate. A - modern soil (control); B - modern soil (chitin); C – soil, buried ~3500 years ago (control); D - soil, buried ~3500 years ago (chitin); E – soil, buried ~4500 years ago (control); F - soil, buried ~4500 years ago (chitin); G – buried permafrost sediments (control); H - buried permafrost sediments (chitin)

OTU 97%	Modern soil		Soil, buried ~3500 years ago		Soil, buried ~4500 years ago		Buried permafrost sediments	
	control	chitin	control	chitin	control	chitin	control	chitin
Shannon index	9.5	6.9	8.5	7.8	7.9	6.3	6.0	5.1
Chao1 index	1755	841	1251	1085	808	751	234	126
Number of species	959	454	715	550	514	336	214	121
PD index	54.9	28.2	43.5	30.4	30.2	19.5	15.7	6.1



Beta diversity of investigated microbiomes (Bray-Curtis dissimilarity) modern soil:
▲ - control ◀ - chitin
soil, buried ~3500 years ago
▶ - control ▼ - chitin
soil, buried ~4500 years ago
⬟ - control ◆ - chitin
buried permafrost sediments
● - control ■ - chitin

RESULTS

Comparison of alpha diversity revealed the expected decrease of all diversity indexes with depth and age of the sample (Shannon index decreased from 9.5 in modern soil to 6.0 in buried permafrost sediments). Also, the alpha diversity was decreased in samples with substrate comparing to control, which indicates the distinguishing of dominant genera. Beta diversity analysis via Bray-Curtis method revealed that hydrolytic complexes of modern soils, buried soils and buried permafrost soils differ from each other and the age of the sample is the main clusterizing factor (statistic analysis was performed with PERMANOVA, $p<0.05$). That fact indicates that different genera perform the substrate degradation in soils of different age. Heatmap analysis of dominant genera revealed the difference in hydrolytic community of the samples.

CONCLUSION

Metagenomics analysis of modern soils, buried paleosoils and buried permafrost sediments revealed the difference in hydrolytic prokaryotic complexes. Due to the fact that previous studies revealed that the intensity of metabolic activity correlated with the age and deposition depth of the sample, the dominant genera of subsurface samples may be considered as potential hydrolytic agents for biotechnology