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**Comparative metagenomic analysis of the hydrolytic procaryotic complexes of modern and buried chestnut soils and buried permafrost soils****Ekaterina Koltsova, Alena Tyapkina and Natalia Manucharova**  
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**Aim:** 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 sub kurgan paleo soils (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 and PRK806R. Analysis of the data was performed in QIIME. OTU picking at the similarity levels 97%, 94%, 91%, 88%, 85%, 81% was performed with use of UCLUST algorithm; all the reads were aligned via PyNAST (Greengenes). Taxonomy was assigned according to RDP classifier and the phylogenetic tree was made with Fast Tree algorithm.

**Results:** 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. 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. In current study we compared alpha and beta diversity in all samples in the presence or absence of substrate. 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 clustering 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 (which are more than 1% of all OTUs at 97%) revealed the difference in hydrolytic community of the samples. In modern soils the dominants of the hydrolytic complex were *Agromyces*, *Bacillus*, *Solibacillus*, *Sphingobium*, *Variovorax*, *Acinetobacter* genera, in buried soils *Saccharothrix*, *Streptomyces*, *Rubrobacter*, *Chitinophaga*, *Brevibacillus*, *Paenibacillus*, *Paenisporosarcina*, *Solibacillus*, *Mycoplana*, *Phenylobacterium*, *Devosia*, *Sphingobium*, *Sphingomonas*, *Achromobacter*, *Janthinobacterium*, *Steroidobacter*, *Lysobacter*, *Thermomonas*, in buried permafrost soils *Brevibacillus*, *Clostridium*, *Sedimentibacter*, *Rhodoplanes*, *Sphingomonas*, *Variovorax*, *Cupriavidus*, *Lysobacter*.

**Conclusion:** We analyzed the alpha and beta diversity of prokaryotic microbiomes of modern soils and paleo soils. Methagenomics analysis of modern soils, buried paleo soils 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. Absolute values of total and active biomass gradually decreased with the increase of deposition depth and age of the soil but the intensity of response to the introduction of the substrate increases with the deposition depth.

**Biography**

Ekaterina Koltsova has completed her Master's degree from Lomonosov Moscow State University in 2013 and she is currently pursuing PhD.

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