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ORIGINAL ARTICLE

The estimation of metagenome and functionally polymorphisms of soil procaryote

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The paper estimates the metagenome as well as the functionally meaningful phylogenetic and taxonomic polymorphisms of orthic blacksoil procaryote in winter wheat agrocoenosis by using the pyrosequencing method. As many as 1708 taxonomic units and 335 procaryote taxons have been detected. The research has determined that the structure of metagenom of orthic black soil procaryote contained two archael and twenty two bacterial phyla, absolute dominants among which belonged to Proteobacteria –79.6 % and Actinobacteria – 12.9 %. The polymorphism of procaryotic taxons was observed on the level of families, herewith the dominating ones were: Alcaligenaceae, Pseudomonadaceae, Solirubrobacteraceae, Gaiellaceae, Nitrososphaeraceae. The paper shows some phylogenetic connections among the main representatives of procaryote metagenome which were formed in orthic black soil of winter wheat agrocoenosis. Thus, the use of pyrosequencing method, beside the estimation of structure and diversity, opens a prospect for studying the functional metagenome component of soil procaryote.

Key words: metagenome, polymorphism, procaryotic taxons, soil, agrocoenosis.

Introduction

Soil is the main source of formation of microorganisms' biological and genetic diversity. Some advances in Molecular Biology development led to the development of the molecular-biological methods of microorganisms identification and to the creation of a phylogenetic system of their classification. Along with that, studying species and functional compositions of mixed cultures as well as of associations of microorganisms has become feasible (Patyka et al., 2009; Gadzalo et al., 2015).

Theoretically, DNA which has been recovered from a soil sample, represents total DNA for all microorganisms and it is a soil metagenome (Wooley, 2010). By means of polymerase chain reaction with corresponding phylogenetic markers there is a chance to localize, detect and study the genes, which code the ribosomal pRNA. It promotes to a further development of different isolates studying, including the types of soil microbal coenosis which are not cultivated (Kolodiazhnyi, Andronov & Patyka, 2014). Such methods resulted in a new direction in Microbiology - Metagenomics (Tringe & Rubin, 2005; Cole et al., 2009).

Phylogenetic research of soil ecosystems showed that the abundance of procaryote species, which were found in one sample, significantly exceeds the amount of known cultivated procaryote (Patyka et al., 2008). Basing on the DNA kinetics reassociation, which was recovered from different soil samples, the amount of procaryote genomes was estimated in a range of from 2000 to 18000 per gr of soil. Thus, the species diversity, which is present in one gr of soil, significantly exceeds the amount of known procaryotes in a procaryote cataloque (16177 species in a taxonomic browser of the National Center of Biotechnological Information) (Patyka et al., 2012; Taran et al., 2014).

The development of sequencing methods of new generation, including the technologies of pyrosequencing, has significantly broadened the capability to study the biodiversity of complex multy-component systems, of soil in particular (Patyka, Patyka & Patyka, 2009). Such analysis allows to detect real phylotype and taxonomic diversity of soil microbiome components, irrespective of their cultivation on nutritive medium. The data received from after the results analysis amount to a few ten thousand of nucleotide sequences per experimental sample (Wooley, 2010; Patyka et al., 2012).

The target of the research was to estimate the metagenome as well as the functionally meaningful polymorphisms of orthic blacksoil procaryote in winter wheat agrocoenosis under different farming systems by using the pyrosequencing method.

Materials and methods

The analysis of orthic black soil procaryote was conducted during a wheat blooming period from the rhizosphere (0–20 cm) in the stationary field research of the Department of Land Husbandry and Herbology of National University of Life and Environmental Sciences of Ukraine. The field research has forseen the combination of three gradations of farming systems on the basis of differential and surface soil cultivation. The systems of land husbandry differ in the level of resources allocation of food elements: industrial (intensive) – 12 t of organic and 300 kg of active substance of mineral fertilizers ($N_{92}P_{100}K_{108}$); ecological – 24 t/ha of organic and $N_{46}P_{49}K_{55}$ of mineral fertilizers; biological – 24 t of organic fertilizers per ha of arable land in crop rotation without chemically synthesised agro-chemicals by using biological plant protection agents (Tanchyk et al., 2011).

The method of pyrosequencing was used to analize metagenome and the taxonomic structure of orthic blacksoil procaryote (Ronaghi, 2004). The method included:

– the recovering of total DNA of soil organisms and its purifying from humic acids were conducted according to recommended practice (Patyka et al., 2009);

– PCR of gene16S rRNA fragments was conducred in an amplifier ThermalCycler T100 (Bio-Rad, USA) using universal primers F515 GTGCCAGCMGCCGCGGTAA and R806 GGACTACVSGGGTATCTAAT and adding oligonucleotide identificators (MID) for each sample as well as for sequences which are necessary in accordance with Roche protocol (Kuczynski et al., 2012);

– sample preparation, PCR emulsion and sequencing were conducted on GS Junior (Roche, USA) device according to producer's recommended practice;

– the amplicon libraries analysis of gene 16S pPHK was made by means of a program module QIIME (version1.7.0). It included the removing of standard sequences and primers from the wealth of evidence; sequences filtration on their quality, nucleotide sequence alignment; sequences selection and classification against OTU (Operational Taxonomic Unit) using the criteria of 97 % likeness; determining the taxonomic structure of microbal complexes and their comperative analysis; determining Shennon variability indices and Chaol saturation (comparison of predicted OTU amount under corresponding parameters of selection with their amount in the experimentally detected samples) (Ganley & Kobayash, 2007).

The phylogenetic dendrogram was made by the method of pairwise clustering (UPGMA) using the programs MEGA 6.06 (Kunin et al., 2008).

Results and discussion

As follows from the results of pyrosequencing of orthic blacksoil samples in winter wheat agrocoenosis, 177384 nucleotide sequences were received. After the removal of standard sequences and primers from the wealth of evidence, as well after sequences filtration on their quality, 20417 nucleotide sequences with an average length of 252 nucleotide pairs were chosen for metagenome analysis.The number of sequences in samples ranged from 2941 to 3792 depending on the experiment variants (Table 1). Aligning and classification of nucleotide sequences enabled to receive 1708 OUT. Herewith, their number in samples ranged from 216 to 333 depending on the experiment variant, that testifies to a high level of detected biodiversity of the soil procaryote.

The attributing of the metagenome nucleotide sequences on their correspondence to taxonomic units allowed to detect 335 procaryote taxons, 1.8 % of which belonged to domain *Archaea* (30 OTU) and 98.2 % – to domain *Bacteria* (1643 OTU), herewith the amount of non-classified bacteria amounted to 18 % (296 OTU).

The Shannon diversity indices were determined in a rate of 3.55–4.54, which testifies to a high rate of multidirectionality of soil and microbial processes, which promote to the formation of corresponding polymorphism of orthic blacksoil procaryote. The Chao saturation indices were 3.5–5.7 times higher than the amount of detected OTU depending on the experiment varant. It testifies to the fact that the level of real biodiversity of orthic blacksoil procaryote several-fold exceeds the experimentally detected one.

Table 1. Technological and ecological diversity indices of orthic blacksoil procaryote in winter wheat agrocoenosis

Experiment variant		Number of sequences	number of OUT	Chao Index	Shannon Index
Industrial system	DC	3252	238	1355.48	4.54
	SC	3441	223	1035.56	3.55
Ecological system	DC	3278	284	1296.84	4.17
	SC	3792	324	1138.17	4.13
Biological system	DC	2941	216	1207.55	3.74
	SC	3713	333	1344.06	4.09

DC – differential cultivation; SC – surface cultivation

Taxonomic analysis of nucleotide sequences showed the availability of the representatives of two archael (*Crenarchaeota, Euryarchaeota*) and 22 bacterial phylum (*Acidobacteria, Actinobacteria, Armatimonadetes, BRC1, Bacteroidetes, Chlamydiae, Chlorobi, Chloroflexi, Cyanobacteria, Elusimicrobia, Fibrobacteres, Firmicutes, Gemmatimonadetes, Nitrospirae, OD1, Planctomycetes, Proteobacteria, TM7, Thermi, Verrucomicrobia, WS3, WYO*) in the structure of metagenome procaryote of orthic blacksoil. Herewith, 67 % of taxons were identified on the level of family and 33 % on the level of genus. Absolute

dominants as for taxons representativeness in the total number of detected procaryote were the phylum *Proteobacteria* – 79.6 % and *Actinobacteria* – 12.9 %.

Topology of distribution of metagenome representatives in soil according to experiment variants was characterised by the shift in the ratio of the main bacterial taxons. A significant differentiation of absolute dominants according to the experiment variants on the level of large taxonomic units (phylum) was not observed. Taxonomic analysis on the family level allowed to detect representativeness polymorphism of procaryote taxons. The dominating families in the structure of procaryote metagenome were *Alcaligenaceae*, *Pseudomonadaceae*, *Solirubrobacteraceae*, *Gaiellaceae*, *Nitrososphaeraceae* (Fig. 1).

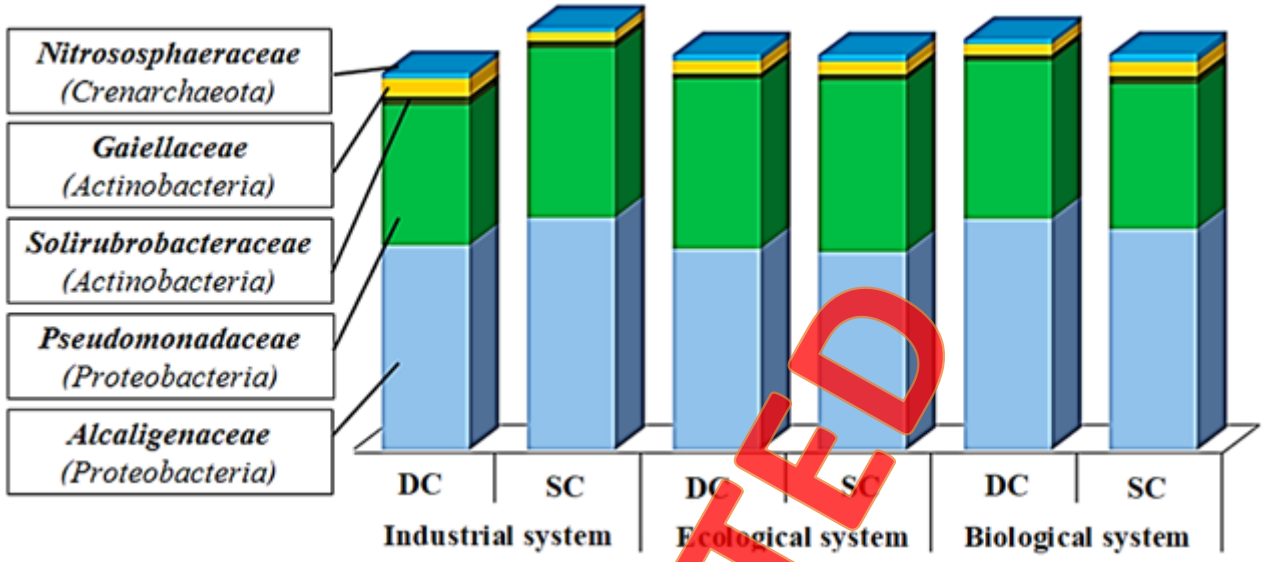

Fig. 1. The distribution of dominating procaryote taxons of orthic blacksoil under different farming systems (DC – differential cultivation; SC – surface cultivation)

A more considerable difference in distribution according to the experiments research was observed among the subdominating microbiome representatives. Herewith, under the biological system of farming were observed both the decrease in the amount of Nocardioidaceae representativeness and the increase in the amount of *Streptomycetaceae* representativeness, as compared to industrial and ecological systems. In the variants with preferred using of organic fertilizers the number of *Xanthomonadaceae*, *Comamonadaceae* families representatives increased and the part of *Bacillaceae* representatives – decreased.

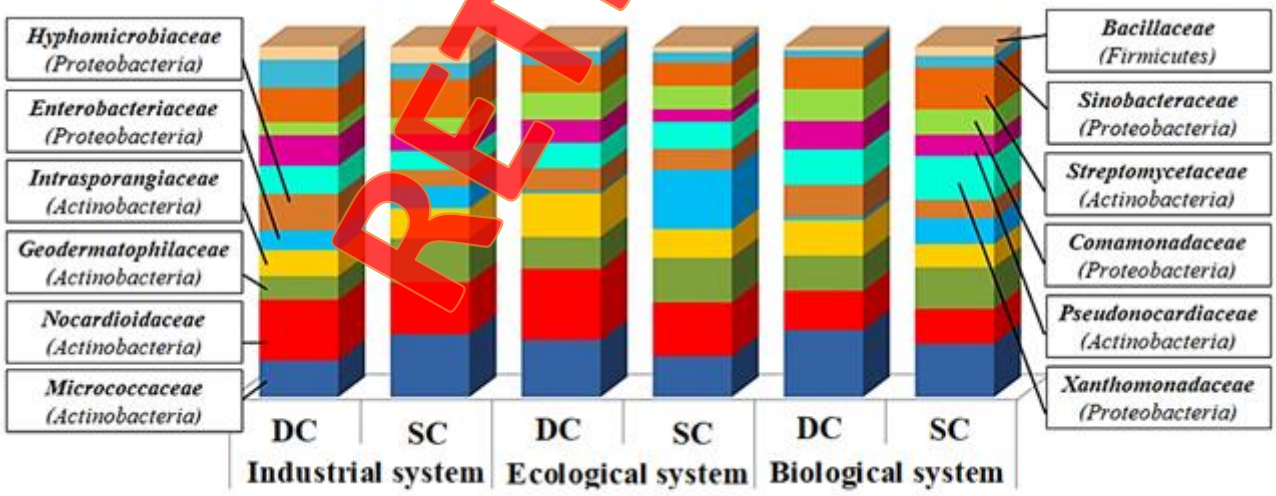

Fig. 2. The distribution of subdominating procaryote taxons of orthic blacksoil under different farming systems (DC – differential cultivation; SC – surface cultivation)

It is necessary to mention that a part of minor procaryote species (the representativeness of each of them did not exceed 0.1 %) totally amounted to16.3–25.2 % depending on the experiment variant, that testifies to their importance in the formation of metagenomic biodiversity. The estimation of biodiversity as well as of the structure of total metagenome of soil procaryote allowed to turn to studying the genetic polymorphism of functionally meaningful dominating taxons. The phylogenetic dendrogram of dominating and subdominating bacterial taxons reflects the distribution topology as well as the evolutional distance among the main representatives of metagenome of orthic blacksoil procaryote in winter wheat agrocoenosis (Fig. 3).

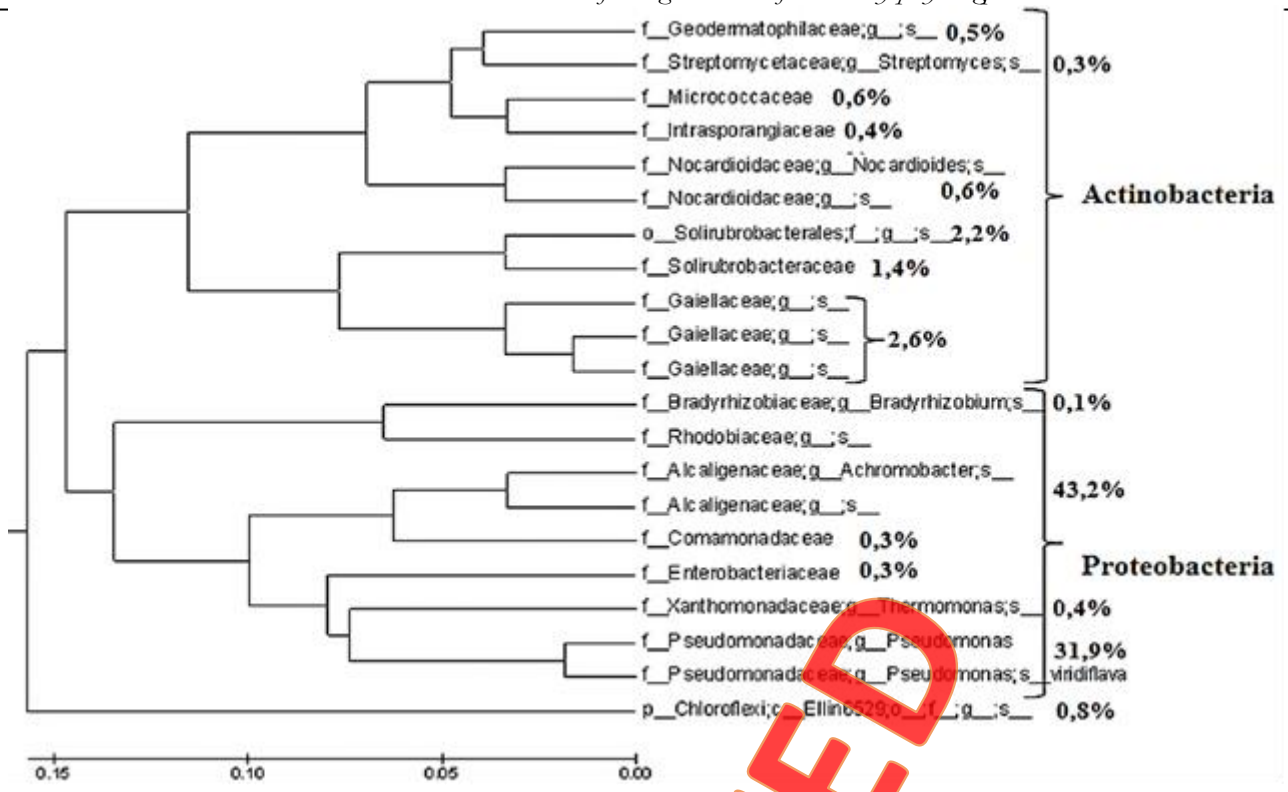

Fig. 3. Genetic polymorphism of functionally meaningful metagenome toxons of orthic blacksoil procaryote in winter wheat agrocoenosis (the dendrogram was formed on the basis of comperative analysis data of nucleotide sequences of 16S pPHK gene of dominating procaryote; (the percents reflect the part of each taxon in total metagenome)

The topology of OTU distribution testifies to the availability of two main clasters which unite the representatives of *Actinobacteria* i *Proteobacteria* phyllum. Thus, the genetic similarity and connection of the main identified procaryote representatives of the soil has been detected.

The absolute dominants, the representatives of *Achromobacter* and *Pseudomonas* genes, are in one claster but in different subclasters. It testifies to the fact that there is some genetic distance between them, which is caused by evolution processes of microbiome formation.The claster of *Actinobacteria* phyla included two subclasters into which, according to the genetic similarity, fell the families representatives: 1 – *Geodermatophilaceae*, *Streptomycetaceae*, *Micrococcaceae*, *Intrasporangiaceae*, *Nocardioidaceae*; 2 – *Gaiellaceae*, *Solirubrobacteraceae*.

The representatives of *Chloroflexi* phyla were relegated to a separate claster which has a rather remote phylogenetic connections.

Conclusions

Thus, the use of pyrosequencing method allowed to estimate again the biodiversity and genetic potential of procaryotic complex of orthic blacksoil under different farming systems. The research has detected a total metagenome, the distribution structure and the representativeness of separate OTU of soil procaryote in winter wheat agrocoenosis. Metagenomic analysis allowed to detect real phylogenetic diversity (1708 OTU), to identify 335 taxons procaryote on the levels beginning from the domain to the genus. It enabled the researchers to study the taxonomic structure of procaryote biom, to detect the most evident dominants (*Achromobacter* and *Pseudomonas*), subdominants and minor taxons.

The detection of a great number of non-classified OTU testifies to localisation capability as well as to studying new genom which are not cultivated on selective media by classical methods of microbiology.

The results of metagenome analysis lead to a unique chance to detect functionally meaningful for soil agrosystems procaryote polymorphisms, to study their genetic potential and phylogenetic connections, that opens the prospects to study the trophic interactions of procaryotic complexes as the constituents of soil biological systems.

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