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POOL AND DIVERSITY OF PROKARYOTES OF CARBONATE CHERNOZEM OF LONG-TERM FIELD EXPERIENCE OF MOLDOVA

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Abstract. For the first time in Moldova, changes in microbiological indicators obtained using polymerase chain reaction (PCR) in carbonate chernozem were studied depending on the type of land use under different anthropogenic loads. The work used soil samples from winter wheat crop rotation of long-term field experiments of different types of use: without fertilizers, mineral and organic background, as well as 75-year-old fallow land . A high diversity of genetic information with a spectrum consisting of 17 phyla was established. According to the International Committee on Prokaryotic Taxonomy (2021), they are new, identified and / or confirmed by metagenomic analysis. The prokaryotic pool varied from 0.01% to 46.53%: Actinomycetota (38.17-46.53%), Pseudomonadota (19.60-27.02%), Bacillota (7.32-22.40%), Bacteroidota (5.31-8.55%), Acidobacteriota (1.94-3.32%), Verrucomicrobiota (1.37-2.09%), Myxococcota (1.09-1.71%), Nitrospirota (0.15-0.65%), Planctomycetota (0.58-0.65%), Gemmatimonadota (0.36-0.58%), Patescibacteria (0.08-0.17%), Cyanobacteriota (0.05-0.08%), Chloroflexota (0.03-0.07%), Fibrobacterota (0.01-0.05%), Abditibacteriota (0.01-0.07%), Bdellovibrionota(0.01-0.011%), and Nitrososphaerota (7.32-22.4%). The first 16 are representatives of the Bacteria domain, and the Nitrososphaerota phylum belongs to the Archaea domain. Based on the results of multiple observations of the content of conserved DNA regions of different phylogenetic groups in the soil, it can be concluded that the prokaryotic community is susceptible to the type of chernozem use. As an alternative to soil conditions, bacteria act in the following sequence: soil without fertilizers → organic background → mineral background → fallow land, and archaea - vice versa.

Keywords: prokaryotes, bacteria and archaea, 16S rAPH gene, metagenomic analysis.

INTRODUCTION

Prokaryotes, whose representatives include two domains – Bacteria and Archaea, are "the basic unit and universal basis of life" [6]. Approximately 5% of the total number of prokaryotes on Earth are concentrated in the soil [4], characterizing the soil environment as the largest reservoir of microbial diversity on Earth. The majority belongs to bacteria, while archaea account for 0.5 to 3.8% of the total number of all prokaryotes. However, the high level of genetic diversity of the soil microworld remained unknown for many years [1]. Only the advent of new methods of molecular ecology of microorganisms revealed that at present, only about 0.1% of the total microbial diversity of the biosphere is taken into account by the cultivation of microorganisms [2].

With the introduction of methods of molecular ecology of microorganisms, science has gained the opportunity to take into account not only cultivated but also uncultivated forms of microorganisms [8, 12]. It is believed that the microbial community is a kind of "mirror" that reflects the features of the habitat, and its diversity represents the hidden potential of the soil microworld [1, 5] in connection with which the metagenomic characteristics of soils are in demand for assessing the sustainability of soil ecosystems under the influence of natural and anthropogenic factors, and interest in such studies is constantly increasing. The soils of Moldova have not been studied from this point of view.

The aim of this study was to study the pool and diversity of prokaryotes of the carbonate chernozem of the Republic of Moldova using quantitative PCR and high-throughput sequencing of 16S rRNA gene libraries.

MATERIALS AND METHODS

The object of the study was microbial communities of carbonate chernozem: light loam with a humus content of 2.5-3.0%, mobile phosphate of 0.8-1.5 mg / 100 g, exchangeable potassium of 18-22 mg / 100 g and carbonates of 1.8-2.2% in the 0-20 cm layer. The studies were carried out in 2022 in a long-term stationary field experiment (founded in 1950) of the Ketrosy scientific and educational farm, district Anenii Noi . Soil samples were collected in the spring from variants with winter wheatof eight-field of crop rotation : 1 - without fertilizers, 2 - with the application of mineral fertilizers, 3 - with the application of organic fertilizers and 4 - fallow land. Fertilizer application rates: total for crop rotation N675P480K480 (N90P60K60 for corn, N120P60K60 for winter wheat before corn and before sunflower, N45P60K60 for sunflower and peas); total for crop rotation 144 t/ha of semi-rotted manure (40 t for corn, 22 t for winter wheat, 20 t for sunflower).

Metagenomic analysis was carried out using modern methods [3, 4] and equipment of the Collective Use Center “Genomic Technologies, Proteomics and Cell Biology” of the All-Russian Research Institute of Agricultural Microbiology, St. Petersburg, Russia. Taxonomic identification of OTUs was performed using the RDP (SILVA) database, the classification was refined using the online database https://www.ncbi.nlm.nih.gov/taxonomy) [9, 10, 11].

RESULTS AND DISCUSSION

A study of long-term used carbonate chernozem soil revealed that its prokaryotic community consists of 17 phylums: *Actinomycetota*, *Pseudomonadota, Bacteroidota, Bacillota, Acidobacteriota, Verrucomicrobiota, Planctomycetota, Myxococcota, Nitrospirota, Gemmatimonadota, Cyanobacteriota, Patescibacteria, Chloroflexota, Fibrobacterota, Abditibacteriota, Bdellovibrionota,* и *Nitrososphaerota* **(Fig. 1).** *The first 16 are representatives of the Bacteria domain, and Nitrososphaerota* belonged to the domain *Archaea*. According to the quantitative assessment of the content of conservative DNA regions of different phylogenetic groups in the soil, it can be concluded that the bacterial community is most responsive to the type of use of chernozems. As an alternative to soil conditions, bacteria act in the following sequence: soil without fertilizers → organic background → mineral background → fallow land, and archaea - vice versa.

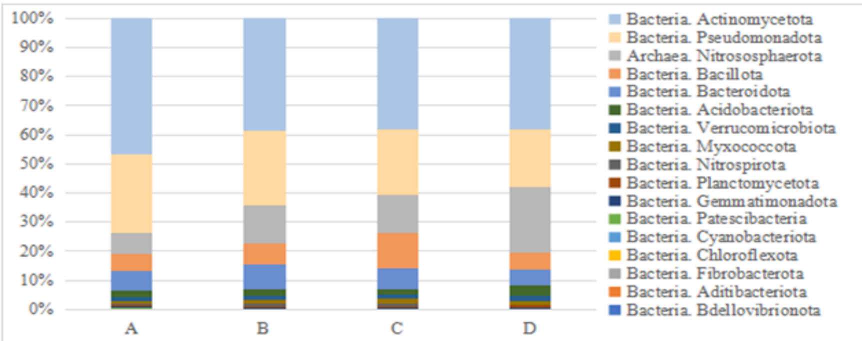


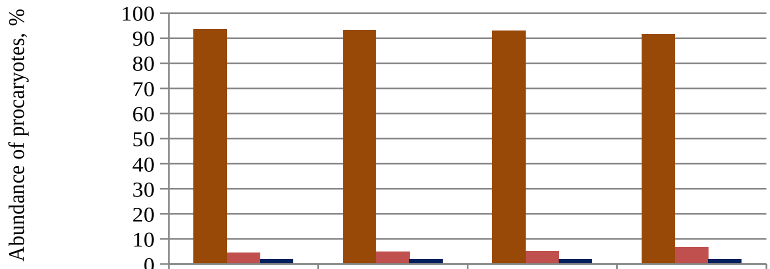
Figure 1. Taxonomic structure of prokaryotic communities of carbonate chernozem at the phylum level, %:
A – without fertilizers. B – mineral background, C – organic background, D – fallow land

The identified microbiome was similar in the composition of its components during the study, but the presence of phyla in the variants was different. All phyla were simultaneously present only in the soil of the crop rotation without fertilizers (control). In the fertilized variants, 15 phyla were identified, and in the fallow soil – only 13. The phyla also differed in their proportion of representation in the community. Among the identified prokaryotes, the highest abundance was recorded in microorganisms of the Bacteria domain (77.60–92.68%). The leaders among them were representatives of the Actinomycetota phylum (38.17–46.53%), reaching the highest value in soil without fertilizers and the lowest in soil fertilized with organic fertilizers for a long time and in the fallow land. Next, among the majority of components of the prokaryotic community, representatives of Pseudomonadota (19.60-27.02%) were widely encountered, which in general were almost 2 times inferior to actinobacteria in numbers, recording a similar development with actinobacteria in variants. The third in terms of representativeness was the archaeal phylum Nitrososphaerota (7.32-22.40%).

despite the fact that it was represented almost twice less than the previous one, it recorded the highest ratios in the fallow and the lowest in the unfertilized soil, while the fertilized variants occupied an intermediate position. In addition, it should be emphasized that the contribution of archaea in the prokaryotic community doubled in fertilized variants (13.04-13.25%) in relation to unfertilized soil (7.32%), while at the same time noting a doubled representation in the fallow land soil (22.40%) in relation to fertilized variants (13.04-13.25%) and an almost 4-fold excess in relation to unfertilized crop rotation soil (7.32%). The phylum Bacillota was also characterized by high rates (6.08.-12.42%), distinguished by the highest values of abundance in the organic background soil and the lowest in unfertilized soil and fallow land.

The Bacteroidota phylum had a taxa abundance of 5.31-8.55% with the highest indicators in the fertilized variants (6.90-8.55%).Further, attention is drawn to the phyla that did not differ in either large fluctuations or large values of abundance in the community: Acidobacteriota (1.94-3.32%) Verrucomicrobiota (1.37-2.09%) and Myxococcota (1.09-1.71%). They recorded the highest indicators in the soil of the organic background or in the fallow soil, and the lowest - in the unfertilized soil. Of these, the highest values were recorded by the phylum Acidobacteriota (3.32-1.94%) and the lowest - by the phylum Myxococcota, and Verrucomicrobiota (1.37-2.09%) occupied an intermediate position among them. The following 9 phyla had very small contributions to abundance prokaryotic community. Moreover, the first 5 phyla: Nitrospirota (0.15-0.65%), Planctomycetota (0.58-0.65%), Gemmatimonadota (0.36-0.58%) Patescibacteria (0.08-0.19%) Fibrobacterota (0.01-0.05%) were found comparatively more often and showed 0.03-0.65% contribution, in comparison with the rest. And the phyla Aditibacteriota and Bdellovibrionota with a contribution of only 0.01-0.07% were not identified in the soil of fertilized variants and in the fallow land soil.

Ranking of phyla by individual “significance” abundence revealed that long-term agricultural use of carbonate chernozem provoked a strong restructuring of the prokaryotic community. The same composition of components hides a distinctive hierarchical role of taxa in the community (Fig. 2). Based on this, all phyla can be divided into 3 groups, which, being constant in composition, differ in their relative abundance throughout the study period. The first group with a dominant ecological role and an occurrence frequency of ≥5% had the highest representation among these 3 groups, individually differing significantly both in the variant and among them, accounting for about 91.56–93.63% of 16S rRNA genes from all sequences without significant differences among variants themselves.It consisted of 5 phyla, which in order of decreasing abundance were as follows: Actinomycetota (38.17–46.53%), Pseudomonadota (19.60–27.02%), Nitrososphaerota (7.32–22.40%), Bacillota (6.08–12.42%) and Bacteroidota (5.31–8.55%). The second group, commonly found commonly encountered, was formed from 3 phyla with a secondary ecological role and abundance ≤ 5% and averaged about 4.40–6.60%, recording an increase of 25% in the organic background soil and 50% in the fallow soil, compared to the rest. Moreover, the greatest contribution of Acidobacteriota (1.94–3.32%) and Verrucomicrobiota (1.37-2.09%) was recorded in the fallow soil, and Myxococcota (1.09-1.71%) in the organic background. The smallest contribution of this group of microorganisms was noted in the unfertilized soil of the crop rotation. Representatives of the second group of prokaryotes formed the following descending sequence: Acidobacteriota (1.94–3.32%) → Verrucomicrobiota (1.37–2.09%) → and → Myxococcota (1.09–1.71%) – in the soilorganic background. The third group included rarely seen representatives of prokaryotes with an abundance of ≤ 1%, having an insignificant ecological role in the community and amounted to only 1.84-1.97%, differing slightly in variants and at the same time constituting the smallest group of prokaryotes in terms of contribution and the largest group among them in terms of the number of phyla (9).



	A	B	C	D
Group ≥5%.	93,63	93,13	92,93	91,56
Group 1-5%	4,4	4,93	5,17	6,6
Group ≤ 1%.	1,97	1,94	1,9	1,84

Figure 2. Redistribution of the relative participation of prokaryotes under winter wheat in carbonate chernozem, according to the degree of dominance (d.d.), %:
A – unfertilized, B – mineral background, C – organic background, G – fallow land

They were arranged in descending order of abundance as follows: Nitrospirota (0.15–0.65%) → Planctomycetota (0.58–0.65%) →

Gemmatimonadota (0.36–0.58%) → Patescibacteria (0.08–0.19%) → Cyanobacteriota (0.05–0.08%) → Chloroflexota (0.03–0.07%) → Fibrobacterota (0.01–0.05%) → Abditibacteriota (0.01–0.011%) and → Bdellovibrionota (0.01–0.07%).

Consequently, under the influence of long-term agricultural use of carbonate chernozem, physicochemical and climatic factors from year to year, since the beginning of time, a significant restructuring and redistribution of the prokaryotic community occurs. Microorganisms, changing the degree of their dominance in the community, losing or acquiring a new ecological status, survive and remain an important component of the microbiome of carbonate chernozem [7, 12]. It is these properties that characterize prokaryotes as structural elements of living matter in the soil, which interact with each other in the struggle for nutrients, thereby ensuring the role of the molecular alphabet of living matter.

CONCLUSION

1. Carbonate chernozem is characterized by high genetic diversity of the most ancient representatives of the living world - prokaryotes, the spectrum of which consists of 17 phyla of both domains, similar to soils of other regions. But at the same time, all were present only in the unfertilized soil of the crop rotation.

2. The pool of prokaryotes ranged from 0.01% to 46.53% with the highest values in the unfertilized soil and the lowest in the organic background and fallow soil. The mineral background occupied an intermediate position among them.

3. According to the estimates of the International Committee on Prokaryotic Taxonomy (2021), the phyla are new, identified and / or confirmed by metagenomic analysis.

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