METAGENOMIC ANALYSIS OF BACTERIAL COMMUNITIES IN THE RHIZOSPHERE OF STIPA CAPILLATA AND CENTAUREA DIFFUSA

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ABSTRACT

The effectiveness of bioremediation of unproductive agricultural lands and marginal lands, not only on the zonal climatic conditions and physico-chemical parameters of soil, but also on the used plants and rhizosphere microorganisms. The use of xerophytic perennial grasses, adapted to the regional climatic conditions and physico-chemical parameters of soils, will improve the efficiency of bioremediation of unproductive agricultural lands and marginal lands. The article presents the results of a comparative analysis of the prokaryotic communities of the rhizosphere of the two representatives of the xerophytes herbs - *Stipa capillata* and *Centaurea diffusa*. We have shown that the dominant position in the prokaryotic communities of the rhizosphere of *Stipa capillata* and *Centaurea diffusa* is the phyla *Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes, Verrucomicrobia, Planctomycetes* and *Chloroflexi*. The microorganisms with cellulolytic activity and the destructors of aromatic compounds included in the composition of prokaryotic communities in the rhizosphere of *Stipa capillata* and *Centaurea diffusa*.

Keywords: *Stipa capillata*, *Centaurea diffusa*, 16s rRNA gene, bioremediation

1. INTRODUCTION

Soil is not only the main means of agricultural production, but also an important component of terrestrial biocenoses, a regulator of the composition of the atmosphere, hydrosphere and a reliable barrier to the migration of pollutants. However, this irreplaceable component of the biosphere undergoes significant degradation as a result of anthropogenic impact.

The Orenburg region, with more than 10 million hectares of agricultural land, is among the ten regions of the Russian Federation, where a significant part of crop and livestock production is produced. At the same time, over the past 10 years, cropland that has left agricultural production due to degradation processes is about 1 million hectares. More than 3 million hectares of croplands are characterized by a low content of humus, with an average annual balance in the croplands of 0.22 tons per hectare. At present, the introduction of organomineral fertilizers has decreased by 10 to 15 times in comparison with 1991, the applied doses of mineral and organic fertilizers do not compensate for the loss of soil nutrients.

It is important to note that the process of restoration of the steppe ecosystem, on soils subjected to degradation processes, is long [1], [2], passing through the formation of the cereal steppe with the predominance of annual and biennial grasses, with their gradual replacement by typical steppe plants [3].

It should be noted that microbial communities of soil play an important role in ecosystem processes: the decomposition of organic substances, the transformation of inorganic compounds and the fixation of nitrogen [4], [5]. Microorganisms are particularly sensitive to changes in soil pH [6], [7], zonality [8], vegetation type [9] and land use system [10]. Proceeding from this, one of the main ways of transition to sustainable development of agrophytocenoses is to maintain the ecological balance of natural microbiocenoses.

However, for this, it is first necessary to study the taxonomic structure of the microbial community associated with this type of soil. With the advent of molecular genetic methods of research, it became possible to conduct large-scale studies of microbiocenoses of various habitats.

Therefore, a comparative analysis of the qualitative composition of microbial soil communities exposed to degradation processes will serve as the basis for phylogenetic mapping and will allow developing scientific and methodological approaches to land reclamation.

The aim of the work was a comparative analysis of the prokaryotic communities of the rhizosphere of two representatives of the steppe landscapes - *Stipa capillata* and *Centaurea diffusa*.

2. MATERIALS AND METODS

2.1. Study site

Studies were carried out on the territory of the Perevolotsky district of the Orenburg region, 10 km south-east of the village of Perevolotsky on the steppe site with coordinates 51°49'N and 54°19'E. The site is located in the subzone of southern chernozems. The climate is sharply continental (with sharp amplitudes of climatic elements in separate months), hot dry summers (average July temperature + 22°C), cold winters (average January temperature -15.8°C), insufficient precipitation (average annual precipitation amount 300-350 mm), air dryness, intensity of evaporation processes, frequent droughts and dry winds. The duration of the snow cover is 136 days, with an average height of 20-25 cm.

2.2. Agrochemical parameters of the soil

The soil cover is represented by chernozem southern weakly humified low-powerful light loamy. The humus content is $3.1 \pm 0.9\%$. Absorption capacity - $23.6 \pm 7.6\%$. The average thickness of the humus horizon is 32.0 ± 3.1 cm. Before the study, soil samples were randomized.

2.3. Study species

Stipa capillata L. (Poaceae) is a perennial grass, endemic to steppe landscapes, growing in dry meadows on nutrient - poor sandy and loamy soils. Its area covers the vast territory of Eurasia and mainly steppes of Russia and Kazakhstan. This is one of the most common plant species of the steppe zone of Orenburg Preduralye.

Centaurea diffusa L. is an annual or biennial plant, usually growing from 10 to 60 cm in height. It has a highly branched stem and a large taproot, as well as a basal rosette of leaves with smaller leaves alternating on vertical stems. Grown in Asia Minor (Turkey, Syria), the Balkans (Bulgaria, Greece, Romania), in Ukraine and southern Russia.

Plant samples were selected at five points of the experimental field 100×100 meters, in early July 2017. Each plant cut off the upper part of the stem, leaving the basal part (5-10 cm). The roots of the plant were dug up together with the soil, placed in plastic bags and delivered to the laboratory. In the laboratory, copies were stored in a refrigerator (at a temperature of - 20°C, 18 hours). To prepare the aqueous extract, the basal soil was cut off together with the roots. The obtained soil samples were combined. Of the common model of a unified land made weighed (10 g) and dissolved in 20 µl TE-buffer (100 mm Tris-HCl, pH=7.5, 20 mm EDTA, 1.4 M NaCl). After bottling left to settle for 60 minutes. Subsequently, aliquots (10 ml) were selected and filtered through membrane filters of 0.45 µm (Millipore, USA). The filters were stored at (-20°C), prior to metagenomic analysis. To determine the species composition of plants, "The determinant of vascular plants of the Orenburg region" was used [11]. Latin names of species are given in summary Cherepanov S.K. [12].

2.4. Isolation of DNA
The samples for the isolation of total DNA were samples of frozen soil (0.5 g each). DNA from the samples was isolated using NucleoSpin®Soil (Macherey-Nagel, GmbH & Co. KG (Dep., IT-EDV) in accordance with the manufacturer's instructions. The purity of the isolated DNA was monitored by photometry on a NanoDrop 8000 instrument (Termo Fisher Scientific Inc., USA) and also by electrophoresis on a 1.5% agarose gel. The DNA concentration in the samples was determined on a fluorometer "Quantus" ("Promega" USA) using a set of Quanti Fluor dsDNA of the same manufacturer. DNA libraries for sequencing were created using the Illumina protocol (http://support.illumina.com/documents/documentation/ chemistry documentation/16s/16s-metagenomic-libraryprep-guide-15044223-b.pdf), using primers to V3 and V4 regions 16S rRNA for prokaryotes (direct SD-Bact-0341-bS-17 and reverse SD-Bact-0785-aA-21). Metagenomics sequencing was performed on a MiSeq sequencer (Illumina, USA) with a set of reagents according to the manufacturer's recommendations at the collective use center for scientific equipment "Persistence of microorganisms" of the Institute of Cellular and Intracellular Symbiosis of the Ural Branch of the Russian Academy of Sciences.

2.5. Bioinformatic analysis
Taxonomic identification of nucleotide sequences and a comparative analysis of microbial communities were carried out using the Internet resource VAMPS (Visualization and Analysis of Microbial Population Structures) available on http://vamps.mbl.edu/. The sequence was sorted using the RDP (Ribosomal Database Project), available at http://rdp.cme.msu.edu/.

3. RESULTS AND DISCUSSION
Metagenomic analysis of bacterial communities in the rhizosphere of *Stipa capillata* and *Centaurea diffusa*
The 16S metagenomic analyses of the rhizospheres *Stipa capillata* shows that the prokaryotic composition were classified into bacteria with 109.2 reads (99.5%), archaea with 96 reads (0.1%) and 439 reads (0.4%) were unclassified at the kingdom level, suspected to belong to the third kingdom (domain), the eucaryota.

Prokaryotic organisms belonging to 27 Phyla were detected using this 16S metagenomic tool. The top 8 of 27 phyla (with the number of reads) belonged to the Phyla *Proteobacteria* (26.9%), *Actinobacteria* (22.1%), *Firmicutes* (12.8%), *Bacteroidetes* (7.0%), *Verrucomicrobia* (5.2%), *Caldithrix* (6.7%) and *Chloroflexi* (1.5%). At this level, 12.0% were unclassified.

A total of 58 classes of prokaryotic organisms were detected from the soil. The top 8 of 58 classes were identified to be *Actinobacteria* (16.2%), *Alphaproteobacteria* (10.6%), *Clostridia* (9.7%), *Caldithrixae* (8.1%), *Sphingobacteriia* (6.6%), *Deltaproteobacteria* (5.6%), *Betaproteobacteria* (4.9%). A total of 14.3% were unclassified.

The 16S metagenomic result of the soli identified and thus classified all the procaryotic organisms present into a total of 230 families. The identities of the top 8 of the 230 families as *Caldithrixaceae* (6.7%), *Chitinophagaceae* (4.6%), *Thermoanaerobacteraceae* (3.4%), *Micromonosporaceae* (3.1%), *Chthoniobacteraceae* (2.7%), *Pseudonocardiaceae* (2.2%), *Sphingomonadaceae* (2.2%). A total of 24.0% were unclassified.

Using the 16S metagenomic tools, a total of 870 prokaryotic species were identified to be present in the soil. The seven most predominant species were identified to be *Caldithrix palaeochoryensis* (5.9%), *Chthoniobacter flavus* (2.5%), *Tepidanaerobacter syntrophicus* (2.5%), *Euzebya tangerina* (1.6%), *Chondromyces pediculatus* (1.3%), *Megasphaera hominis* (1.2%) and *Segetibacter aerophilus* (1.0%). Of this 870 species, 56.8% of the species were considered unclassified.

In summary, the prokaryotic composition of the soil was so diverse that 870 different species, 519 genera, 230 families, 111 orders, 58 classes, 27 phyla were identified using the 16S metagenomic tools as compared to the results obtained using the culture-dependent techniques.

The 16S metagenomic analyses of the rhizospheres *Centaurea diffusa* shows that the prokaryotic composition were classified into bacteria with 122.534 reads (98.7%), archaea with 53 reads (0.04%) and 1.563 reads (1.26%) were unclassified at the kingdom level, suspected to belong to the third kingdom (domain), the eucaryota.

Prokaryotic organisms belonging to 29 Phyla were detected using this 16S metagenomic tool. The top 8 of 29 phyla (with the number of reads) belonged to the Phyla *Proteobacteria* (38.1%), *Actinobacteria* (24.7%), *Firmicutes* (10.8%), *Bacteroidetes* (6.8%), *Verrucomicrobia* (2.6%), *Caldithrix* (2.3%) and *Planctomycetes* (1.3%). At this level, 10.2% were unclassified.

A total of 59 classes of prokaryotic organisms were detected from the soil. The top 8 of 59 classes were identified to be *Actinobacteria* (20.0%), *Alphaproteobacteria* (16.2%), *Gammaproteobacteria* (11.7%), *Clostridia* (6.9%), *Sphingobacteriia* (6.0%), *Betaproteobacteria* (4.7%) and *Deltaproteobacteria* (4.4%). A total of 12.5% were unclassified.

The 16S metagenomic result of the soli identified and thus classified all the procaryotic microorganisms present into a total of 235 families. The identities of the top 8 of the 235 families as *Sphingomonadaceae* (3.6%), *Chitinophagaceae* (3.5%), *Pseudonocardiaceae* (3.5%), *Caldithrixaceae* (2.3%), *Coxiellaceae* (3.3%), *Micromonosporaceae* (3.0%) and *Rhodospirillaceae* (2.5%). A total of 21.6% were unclassified.

Using the 16S metagenomic tools, a total of 1186 prokaryotic species were identified to be present in the soil. The seven most predominant species were identified to be *Caldithrix palaeochoryensis* (2.0%), *Tepidanaerobacter syntrophicus* (1.5%), *Chthoniobacter flavus*

(1.3%), *Chondromyces pediculatus* (1.0%), *Aquicella siphonis* (1.0%), *Megasphaera hominis* (1.0%) and *Aquicella lusitana* (1.0%). Of this 1186 species, 57.2% of the species were considered unclassified.

In summary, the prokaryotic composition of the soil was so diverse that 1186 different species, 576 genera, 235 families, 111 orders, 59 classes, 29 phyla were identified using the 16S metagenomic tools as compared to the results obtained using the culture-dependent techniques.

Phylogenetic analysis of the bacterial communities of the rhizosphere *Stipa capillata* and *Centaurea diffusa* showed that the dominant groups of microorganisms are of the *Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes, Verrucomicrobia, Planctomycetes* and *Chloroflexi*. Differences in the composition of the bacterial communities of the rhizosphere of the plants. A large proportion of the bacterial communities of the *Stipa capillata* and *Centaurea diffusa* rhizosphere were occupied by the cellulosolytics (the families *Micromonosporaceae, Chitinophagaceae, Chthoniobacteraceae*). The bacteria of the family *Chitinophagaceae* possess the ability of the enzymatic cleavage of chitin and cellobiose in soil [15] and the rhizosphere of plants [16].

In addition, representatives of the *Sphingomonadaceae* family (aromatic decomposers) were present in the bacterial communities of the *Stipa capillata* and *Centaurea diffusa* rhizosphere. The presence of *Sphingomonadaceae* family members in the bacterial communities, not only contributes to the effective destruction of aromatic compounds in the course of remediation of contaminated soils, but also is a powerful weapon in the competitive struggle against "invasive" plant species that can release allopathic compounds.

4. CONCLUSION

The high adaptation of the weeds of *Centaurea diffusa* to growth under extreme conditions is often associated with the composition of the bacterial community of the rhizosphere of the plant. A comparative analysis of the composition of bacterial communities of the rhizosphere *Stipa capillata* and *Centaurea diffusa* showed their similarity in the main groups of microorganisms. That, application of xerophytic turf grass *Stipa capillata* for bioremediation of unproductive agricultural lands and marginal lands will contribute to its effectiveness.

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