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Original Article

Features of the Formation of Communities of Microorganisms Adapting to the Existing Conditions in Different Types of Agrophytocenoses

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Abstract

The article is devoted to studying the features of the formation of communities of microorganisms, adapting to the conditions of existence in various types of agrophytocenoses. The study was carried out on model weed plant species, the most common in agrophytocenoses of the Belgorod region. Two nutrient media were prepared for microbiological examination: nutrient agar (BPA - the number of mesophilic aerobic and facultative anaerobic microorganisms) and ENDO - an elective medium for CB (*Escherichia coli* bacteria). It was found that up to 80% of the total numbers of epiphytes are *Erwinia herbicola* (*Pseudomonas herbicola*) cells. The most common among the epiphytic bacteria of terrestrial higher weeds are traditional representatives of cosmopolitan bacteria such as *Pseudomonas fluorescens, Ps. furbicolaaurum, Ps. putida, Pantoea agglomerans, Arthrobacter flavescens, A. album, Lactobacillus Plantarum, Bacillus subtilis, B. megaterium, Klebsiella rosea, Agrobacterium.*

Keywords: Microorganisms, Agrophytocenoses, Nutrient medium

1. Introduction

Our floristic research carried out in the south-west of the Central Russian Upland, which we consider within the administrative boundaries of the Belgorod region, identified and studied various types of anthropogenic transformation of the region's flora: urban flora, man-made ecotope flora, including railways, the flora of agrophytocenoses, the flora of natural-reserved fund. These florae are formed during the parallel evolution of the vegetation cover in the Anthropocene against the background of the development of steppe vegetation under a relatively intense anthropogenic influence (1).

One of the most dynamic and peculiar types of anthropogenic transformation of flora is the flora of agrophytocenoses, which, due to the significant plowing of steppe habitats, the presence of a large number of adventive and weed species (2, 3) occupies significant territories in the south-west of the Central Russian Upland. Currently, based on the analysis of floristic characteristics, we have detailed the classification and factors of the formation of the flora of agrophytocenoses as one of the types of anthropogenically transformed flora of the region (4). A summary of the segetal flora of the Belgorod region has been compiled, including 326 species of higher plants. At the same time, the study of the flora of agrophytocenoses in relation to the presence of the most widespread, dominant microorganisms in crop weeds has practically not been studied, despite their important role in the growth and development of both weed and crop species. In this regard, we tried to

conduct a study on identifying microorganisms on model species of weeds in the region.

2. Materials and Methods

The work was carried out at USI "Botanical Garden of the National Research University" BelSU" in the framework of the implementation of the world-class REC program "Innovative solutions in the agro-industrial complex."

We studied the features of the formation of communities of microorganisms, adapting to the conditions of existence in various types of agrophytocenoses on model weed plant species, the most common in agrophytocenoses of the region. For microbiological research, two nutrient media were prepared: nutrient agar (BPA - the number of mesophilic aerobic and facultative anaerobic microorganisms) and ENDO - an elective medium for CB (Escherichia coli bacteria) (5).

ENDO is a differential diagnostic medium designed to isolate and detect *Enterobacterium* and *Escherichia*. Distilled water (150 ml) was poured into a conical flask, and 6.7 g of ENDO medium was added. The medium amount was calculated according to the proportion: 1 L of distilled water requires 40 g of ENDO. The flask was sealed with a stopper, tied with parchment paper, heated on a hotplate until boiling (the medium turns red), and autoclaved at 121 °C for 20 min. For its complete preparation. Then, while still hot, it was poured into Petri dishes.

To prepare nutrient agar (BPA), 150 ml of running water was poured into a conical flask, and 4.5 g of the medium was added. Gradually add the medium to the flask, and stir slowly to avoid clumping. The culture medium was autoclaved at 121 °C for 20 min.

The sterilized culture medium was poured under a sterile fume hood into previously prepared Petri dishes, which were also sterilized in a drying cabinet at 140 °C within 2.5 hours. The culture medium was evenly poured into the dishes, ensuring the entire bottom was covered.

Identification of bacteria was carried out using the Bergey identifier (6).

3. Results and Discussion

A particular experiment was carried out to study the epiphytic microflora. 150 ml of distilled water was used, 5 ml of which was poured into 2 bottles. Then, swabs of the most common model plant species in agrophytocenoses, such as *Amaránthus retroflexus* L., *Erigeron canadensis* L. *Cyclachaena xanthiifolia* (Nutt.) Fresen was obtained with sterile tweezers and cotton wool over the funnel. The cotton wool was left in a weighing bottle; then, according to the method, the solution was diluted, and 12 test tubes were prepared with poured autoclaved water (9 ml in each). In the first tube, 1 ml of the solution from the first bottle was added and mixed well. Then 1 ml of solution was taken from the first tube and added to the second tube; with the same sequence, the solution was diluted six times. The result was a dilution: in test tube No.6 – 1:1,000,000, in test tube No.5 – 1:100,000, in test tube No.4 – 1:10,000.

0.5 ml of solution was taken with a sterile syringe from tube 6, and microorganisms were inoculated onto a Petri dish with BPA and ENDO medium (three dishes each). The result was a 1:1,000,000 dilution. The rest of the dilutions were done in the same way.

All inoculated Petri dishes were placed in a thermostat at a temperature of 28 °C for five days. After this time, the number of colonies grown on nutrient media was determined.

Epiphytic microorganisms, multiplying on the surface of plants, create a biological barrier that prevents the penetration of parasites into plant tissues (7). Enhancing the proliferation of epiphytic microflora by spraying plants with nutrient solutions improves the antagonistic impact of epiphytes against phytopathogenic microbes, indicating that certain plant diseases can be fought by acting on their epiphytic microflora (8).

Up to 80% of the total number of epiphytes are *Pseudomonas* cells (Figure 1). This non-spore-forming bacterium on meat-peptone agar forms golden-yellow colonies. Epiphytic microorganisms on a healthy plant are primarily related to the climate. In wet weather, their number increases, while in dry weather, on the contrary, it decreases. Those plants that more

intensively secrete metabolic products on the surface of tissues had richer and more diverse microflora.

Cell counting on Petri dishes was performed by fluorescence microscopy after staining with methylene blue (Table 1). Photographs were taken to observe and identify microbial landscapes on the surface of the Petri dishes. Microscopic examination of the swabs revealed that the microbial community of the phyllosphere surface is represented by single cells and their clusters in the form of microcolonies, which indicates the growth of bacteria. Morphologically, bacterial diversity is represented by cocci, single and chains, and numerous straight and curved (arcuate) bacilli of various sizes.

The most common among the epiphytic bacteria of terrestrial higher weeds by species composition are traditional representatives of cosmopolitan bacteria such as *Pseudomonas fluorescens, Ps. furbicolaaurum, Ps. putida, Pantoea agglomerans, Arthrobacter flavescens, A. album, Lactobacillus Plantarum, Bacillus subtilis, B. megaterium, Klebsiella rosea, Agrobacterium* (9, 10).

The number of epiphytic microorganisms at the beginning of the growing season is much lower. In the MPA medium, it averaged 10^6 (273.66), 10^5 (498.33), 10^4 (437.26) dilution; in Levin's medium, (124.00), (246.33), and (536.66), respectively; in Czapek's media, isolated fungi, typical epiphytes, which are present in the air, were noted; their spores are easily carried by the wind and settle on the surface. The identified fungi at the beginning of the growing season belong to the genera *Aspergillus* and *Penicillium*.

We found that up to 80 % of the total number of epiphytes are *Erwinia herbicola* (*Pseudomonas herbicola*) cells. The most common among the epiphytic bacteria of terrestrial higher weeds are traditional representatives of cosmopolitan bacteria such as *Pseudomonas fluorescens, Ps. furbicolaaurum, Ps. putida, Pantoea agglomerans, Arthrobacter flavescens, A. album, Lactobacillus Plantarum, Bacillus subtilis, B. megaterium, Klebsiella rosea, Agrobacterium.*

Figure 1. *Pseudomonas*

Table 1. The number of colonies of microorganisms in a Petri dish at a 10^6 dilution in different types of agrophytocenoses

Species Medium	MPA			CZAPEK			LEVİN		
Amaranthus retroflehsus									
Farms	284	293	244	-	-	-	111	134	127
Agricultural holdings	236	154	216	1	1	2	60	74	69
Erigeron canadensis									
Farms	144	86	98	2	1	3	142	120	100
Agricultural holdings	-	-	-	-	-	-	-	10	15
Cyclachaena xanthiifolia									
Farms	253	261	234	-	-	-	98	112	107
Agricultural holdings	221	233	203	-	-	-	-	-	-

Authors' Contribution

Study concept and design: V. K. T.
Acquisition of data: V. N. Z.
Analysis and interpretation of data: E. N. D.
Drafting of the manuscript: M. Y. T.
Critical revision of the manuscript for important intellectual content: L. A. T.
Statistical analysis: L. A. T.
Administrative, technical, and material support: V. K. T.

Conflict of Interest

The authors declare that they have no conflict of interest.

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