

ECOLOGICAL ASPECTS OF BIOSECURITY IN MODERN AGROECOSYSTEMS

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ABSTRACT

Antibiotic resistance is a serious threat to human health and biosecurity across the world. The soil microbiome plays an important role in the development and spread of antibiotic resistance in humans. The aim of this study was to detect the antibiotic resistance soil bacteria in different agroecosystems. We have isolated 244 dominating bacteria; among of them 53 antibiotic-resistant strains had been detected. The most isolates belonged to multi-drug resistant strains, greater than 62,3% of which were resistant to 9 antibiotics. A study of the agroecosystems where *Capsicum annuum*, *Vitis vinifera*, *Rubus idaeus* L., *Petroselinum crispum* were cultivated showed that the microbial community of soil samples have been characterized by a high content of antibiotic-resistant microorganisms. From the soil were isolated antibiotic resistant anaerobic and aerobic microorganisms: *Clostridium perfringens*, *Clostridium oedematiens*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Hafnia alvei*, *Bacillus megaterium*, *Bacillus mycoides*, and *Pseudomonas aeruginosa*. Modern agroecosystems are the source of spread of pathogenic and conventionally pathogenic microorganisms with detected multiple antibiotic resistances and potentially endangering of human health.

Keywords: agroecosystem, antibiotic resistance, biosecurity, microbiome, soil.

INTRODUCTION

Soil plays a vital role in ecosystems, serving as the primary nutrient base and habitat for plants and organisms, and functioning as a giant bioreactor for degrading pollutants and facilitating nutrient transformation. Inevitably, soil is a hot spot for antibiotics to affect indigenous microbes since it receives a large portion of excreted antibiotics through application of manure and sewage sludge as fertilizers (Thiele-Bruhn, 2003; Symochko 2017). The higher density of microbes in the soil environment encourages genetic exchanges, which could enhance the development of microbial resistance in the presence of antibiotics (Martínez, 2008; Symochko et al, 2018).

Environmental contamination by antibiotic compounds is inextricably linked to development of antimicrobial resistance in non-target species of bacteria. Whether the bacteria perform critical ecosystem services, pose a health threat as pathogens or have incompletely understood functions in nature, development of antimicrobial resistance as

a result of human activities is problematic. Susceptibility characteristics of microbes can be altered by incorporation of genetic information encoding for resistance or by mutation in their DNA. Antibiotic resistance genes are recognized as important environmental contaminants (Kümmerer, 2004; Pruden et al, 2006; Wright, 2010; Symochko et al, 2019). More and more evidence is being collected to support the idea that the environment acts both as a reservoir for antibiotic resistance and a means by which this resistance can be broadly disseminated (Symochko, 2017). Antibiotics used in therapeutic doses on farms for pigs and cows, together with their feces, are subsequently exported to fields with agricultural plants. Even in small doses, they can cause a toxic effect and form antibiotic resistance of microorganisms (Boxall et al, 2006; Symochko et al, 2019).

Food consumption represents only one exposure route of humans to environmental antibiotic resistance and also for the interaction of humans with the natural resistome (Sannes et al, 2008). The relative risks of environmental, community-acquired, and nosocomial exposure are largely unknown and ideally would be considered in a human health risk assessment framework (Robinson et al, 2010; Zhang et al, 2011; Symochko, 2019). Large amounts of manure have been applied to arable soils as fertilizer worldwide. Manure is often contaminated with veterinary antibiotics which enter the soil together with antibiotic resistant bacteria. However, little information is available regarding the main responders of bacterial communities in soil affected by repeated inputs of antibiotics via manure (Hammesfahr et al, 2008; Heuer, 2011). Nevertheless, some investigators have commented that antibiotic resistance transfer via vegetables represents a risk to human health. Estimation of food security in different type of agroecosystems and screening of antibiotic-resistant bacteria-causative agents of human diseases is one of the important aspects in modern agroecology and environmental microbiology.

MATERIALS AND METHODS

Materials of research were soil samples, which had been collected by envelope method from agroecosystems: with cultivated of *Capsicum annum*, *Vitis vinifera*, *Rubus idaeus* L., *Petroselinum crispum*, (organic fertilizer has been used continuously for the last 3 years). Studies of soils were carried out at the Scientific Research and Educational Center of Molecular Microbiology and the Immunology of Mucous Membranes (Uzhhorod National University), Research Laboratory Monitoring of Water and Terrestrial Ecosystems, faculty of Biology (Uzhhorod National University). The research was carried out within the framework of the complex theme «Eco-microbiological monitoring of various types ecosystems of the Carpathian region» №0116U003331 (state registration number), following the standard protocol (Tepper, 2004; Shyrobokov, 2011; Goldman, Green, 2015).

All soil samples were analyzed within 24 hours. Microbiological study of soil was performed in sterile conditions. The method of serial dilution was used to obtain the suspension where microorganisms titre were 10^{-3} CFU/ml. - 10^{-5} CFU/ml. 100 μ l (CFU-Colony Forming Units) of the soil suspension was evenly distributed on the surface of the medium with a sterile spatula. For the study we used the following media: Endos agar, Meat peptone agar, Strepto agar and Entero agar, Agar-Agar, Eshbi agar, Soil agar, Chapek agar, Starch agar, Fedorova Agar, Vinogradsky Agar, Ashbys agar in 4 repetitions. Petri dishes with study material were incubated in the thermostat at 37°C for 48 hours in aerobic and anaerobic (Wilson-Blair Agar, Vinogradsky Agar) conditions. Petri dishes with Czapek agar were incubated in the thermostat at 28°C for 96 hours. All isolated microorganisms were identified by applying of appropriate biochemical test-systems LACHEMA according to the instructions. Antibiotic resistance of the identified microorganisms was analysed by Kirby-Bauer method with the aim to find antibiotic resistant strains of pathogenic microorganisms. A total of 244 isolates from soil of medicinal plants were examined for resistance to 12 antibiotics of the main pharmacological groups: TE₃₀ Tetracycline; VA₃₀ Vancomycin; L₁₀ Lincomycin; CXM₃₀ Cefuroxime; AMP₁₀ Ampicillin; CIP₅ Ciprofloxacin; GEN₁₀ Gentamicin; DO₃₀ Doxycycline; AK₃₀ Amikacin; AMX₁₀ Amoxicillin; E₁₅ Erythromycin; OL₁₅ Oleandomycin. Anaerobic microbiota were additionally tested to Metronidazole MT₅; Rifampicin RIF₅; Clindamycin CD₂. The results of the experimental studies were statistically analyzed using the Microsoft Excel program package. Results were expressed as means (\pm) standard deviation (SD) and (SSD05) smallest significant differences of experiments conducted in quadruplicating. The level of significance selected for the study was $P < 0.05$ (Bailey, 1995).

RESULTS

Environment surrounding is a huge bacterial reservoir, and antibiotic resistance can be passed between bacteria in the environment, including in the soil. Microbiological soil control is necessary for assessing and predicting the possibility of spreading antibiotic-resistant microorganisms.

Soil microbial communities are heterogeneous entities with distinct components that are each capable of responding differently to environmental characteristics. Microbial composition was shown seasonal dynamics and differences in the structure of microbial communities, depending on the time of fertilization. Table 1 show the results of soil studies, where was observed the post-effect of organic fertilizer application. The soil of the agroecosystems was characterized by a high content of ammonifiers and nitrogen-fixing microbiota.

The process of nitrogen fixation was most active in the agroecosystem where was grown *Capsicum annuum*. The number of micromycetes, oligotrophes and pedotrophes was varied in different agroecosystems.

The agroecosystem, on which the *Rubus idaeus* L was cultivated, had been recognised as the one of the studied where the maximum content of oligotrophs and pedotrophs was observed. Their number was respectively 3,46 CFU/ per 1 g/d.s. and 2,52 CFU/ per 1 g/d.s. Long-term application of organic fertilizers contributed to the preservation of positive dynamics in the structure of microbial communities. This is confirmed by a high content of organotrophes in the soil under all plants and a relatively low level of oligotrophy of the soil throughout the entire vegetative period.

One of the important indicators of the ecological and sanitary state of the soil and the whole ecosystem is the presence of conditionally pathogenic and pathogenic microorganisms.

Table 1. The number of different ecological-trophic groups of soil microorganisms in agroecosystems

| № | Agroeco-system | CFU-colony forming units/ per 1 gram of dry soil | | | | | Nitrogen fixing bacteria |
|---|----------------------------|--|------------------|------------------|------------------|--|--------------------------|
| | | Micromycetes | Ammonifiers | Oligotrophes | Pedotrophes | Bacteria using mineral forms of nitrogen | |
| | | *10 ³ | *10 ⁶ | *10 ⁶ | *10 ⁶ | *10 ⁶ | |
| 1 | <i>Capsicum annuum</i> | 16,22 | 6,17 | 1,67 | 1,68 | 5,33 | 77,3 |
| 2 | <i>Vitis vinifera</i> | 28,56 | 4,30 | 2,33 | 2,88 | 3,11 | 50,2 |
| 3 | <i>Rubus idaeus</i> . L | 25,42 | 3,42 | 3,46 | 2,52 | 2,86 | 41,6 |
| 4 | <i>Petroselinu crispum</i> | 13,19 | 7,70 | 1,72 | 1,34 | 4,15 | 64,2 |
| - | SSD ₀₅ | 0,22 | 0,31 | 0,27 | 0,19 | 0,54 | 1,27 |

Note: the data are statistically significant, $P < 0.05$, $x \pm SD$, $n = 4$.

Particularly dangerous are the antibiotic-resistant microorganisms, which, together with the bio production, can enter to the human and animals from the modern agroecosystems (see Table 2).

TABLE 2: Microorganisms resistant to tested antibiotics isolated from soil of model ecosystems

| Agroecosystem | Antibiotic resistant microorganisms (ARM) | Total number of ARM |
|----------------------|---|---------------------|
| Capsicum annuum | Enterococcus faecalis, Yersinia enterocolitica, Bacillus cereus, Bacillus megaterium | 4 |
| Vitis vinifera | Clostridium perfringens, Clostridium difficile, Escherichia coli, Enterococcus faecalis, Bacillus megaterium, Bacillus subtilis, Bacillus cereus, Bacillus licheniformis, Enterobacter cloacae, Serratia fonticola, Pantoea agglomerans, Hafnia Alvei | 12 |
| Rubus idaeus. L | Clostridium perfringens, Clostridium oedematiens, Enterobacter cloacae, Enterococcus faecalis, Hafnia Alvei, Bacillus megaterium, Bacillus mycoides, Bacillus subtilis, Bacillus cereus, Escherichia coli | 11 |
| Petroselinum crispum | Bacillus megaterium, Bacillus licheniformis, Hafnia alvei, Bacillus cereus, Enterococcus faecalis | 5 |

The structure of microbial communities of the soil is interrelated with the presence of antibiotic-resistant pathogenic microorganisms. In the soil of agroecosystems where the number of pedotrophes and oligotrophes was higher, a greater number of antibiotic-resistant microorganisms were isolated. From the agroecosystem where have been cultivated raspberry (*Rubus idaeus. L.*) following antibiotic resistant anaerobic microorganisms were isolated: *Clostridium perfringens* (resistant to erythromycin, clindamycin, tetracycline, rifampicin, amoxicillin, moderately sensitive to metronidazole and vancomycin sensitive), *Clostridium oedematiens* (moderately susceptible to amoxicillin and vancomycin), *Clostridium difficile* (sensitive to metronidazole). Nevertheless, the enrichments of *Clostridium* in soil which was continually treated with manure containing can be dangerous for public health. The enrichment of these bacteria, which are phylogenetically closely related to human pathogens, may improve the chance of transferring antibiotic resistance genes to human pathogens, since horizontal gene transfer is more prevalent between closely related organisms than between those distantly related (Forsberg et al, 2012). Soil particles carrying viable bacteria can be transported over long distances and might contribute to the spreading of antibiotic resistant bacteria over wide geographic ranges (Prospero et al, 2005; Symochko, 2020). A significant number of aerobic microorganisms with multiple antibiotic resistances were isolated from the agroecosystem of raspberry: *Enterobacter cloacae*, *Enterococcus faecalis*, *Hafnia alvei*, *Bacillus megaterium*, *Bacillus mycoides*, and *Pseudomonas aeruginosa*. From the agroecosystem of parsley were isolated mostly bacteria of the genus *Bacillus*, which are rather typical representatives of soil microbiota. Nevertheless, all of them were characterized as antibiotic resistant strains and some isolated microorganisms (*Enterobacter cloacae*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa*) are the causative agents of opportunistic infections. The soil microbiome plays an important role in the development and spread of antibiotic resistance in humans. The risk to antibiotic resistance exposure via produce consumption should best be managed by ensuring that practices designed to protect bioproduction from contamination with pathogenic microorganisms are also protective with respect to exposure to antibiotic-resistant bacteria selected for in the digestive tract of animals or humans.

CONCLUSION

Soil microbiome of agroecosystems with growing plants: *Capsicum annuum*, *Vitis vinifera*, *Rubus idaeus L.*, *Petroselinum crispum* still characterized by high functional biodiversity. The taxonomic structure of microbial community has been determined by biochemical markers and showed significant difference between plant species.

The screening of conditionally pathogenic and pathogenic microorganisms of soil microbiome has proved that modern agroecosystems are the source of the spread of pathogenic and opportunistic antibiotic resistant microorganisms. Around 22% of microorganisms isolated from total amount of dominating bacteria representing of typical soil microbiota communities had been characterized by high level of antibiotic resistance. All of them were defined as pathogenic or potentially pathogenic bacteria and could potentially act as the agents of various human opportunistic diseases.

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