

Food Safety in Agroecosystems-Soil Resistome

Lyudmyla Symochko

Abstract—The World Health Organization has identified antibiotic resistance as a serious threat to human health 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. Were isolated 244 dominating bacteria, among them 53 antibiotic-resistant bacteria. All isolates were multi-drug resistant, of which greater than 62,3% were resistant to 9 antibiotics. A study of soil samples from agroecosystems of *Capsicum annuum*, *Vitis vinifera*, *Rubus idaeus L.*, *Petroselinum crispum* showed that the microbial community characterized by a high content of antibiotic-resistant microorganisms. From the soil were isolated antibiotic resistance anaerobic and aerobic microorganisms: *Clostridium perfringens*, *Clostridium oedematiens*, *Clostridium difficile*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Hafnia alvei*, *Bacillus megaterium*, *Bacillus mycoides*, and *Pseudomonas aeruginosa*. Modern agroecosystems are the source of spread of pathogenic and opportunistic microorganisms with multiple antibiotic resistances and endangering human health.

Keywords— agroecosystem, antibiotic resistance, microbiome, soil.

I. 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 [1]. 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 [2]. 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 [3]–[5]. 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 [6]. 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 [7]. Food consumption represents only one exposure route of humans to environmental antibiotic resistance and also for the interaction of humans with the natural resistome [8]. 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 [9]–[11]. 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 [12]–[15]. 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.

II. MATERIALS AND METHODS

Materials of research were soil samples, which had been collected by envelope method from agroecosystems: with cultivated of *Capsicum annuum*, *Vitis vinifera*, *Rubus idaeus L.*, *Petroselinum crispum*, (organic fertilizer has been used continuously for the last 3 years). Microbiological studies of soil were carried out at the Scientific Research and Educational Center of Molecular Microbiology and the Immunology of Mucous Membranes following the standard protocol [16]. 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 µl 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 in 4 repetitions. Petri dishes with study material were incubated in the thermostat at 37°C for 48 hours in aerobic conditions. The chromogenic selected media Uri-Select 4 (Producer: Bio-Rad, France) was used for isolation of microorganisms in aerobic conditions.

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

Lyudmyla Symochko is with the Uzhhorod National University, Uzhhorod, Ukraine.

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. Results were expressed as means (\pm) standard deviation (SD) and (SSD05) smallest significant differences of experiments conducted in quadruplication. Data were evaluated using the software Statistica 7.0.

III. 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.

TABLE I: THE NUMBER OF DIFFERENT ECOLOGICAL-TROPHIC GROUPS OF SOIL MICROORGANISMS IN AGROECOSYSTEMS

№	Agroecosystem	CFU-colony forming units/ per 1 gram of dry soil					%
		I	II	III	IX	X	
		*10 ³	*10 ⁶	*10 ⁶	*10 ⁶	*10 ⁶	-
1	Capsicum annum	16,22	6,17	1,67	1,68	5,33	77,3
2	Vitis vinifera	28,56	4,30	2,33	2,88	3,11	50,2
3	Rubus idaeus. L	25,42	3,42	3,46	2,52	2,86	41,6
4	Petroselinum crispum	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

* I Micromycetes; II Ammonifiers; III Oligotrophes; IX Pedotrophes; X Bacteria using mineral forms of nitrogen; XI Nitrogen fixing bacteria.

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

In the agroecosystem, where *Rubus idaeus. L* was cultivated, 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 through out 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. Particularly dangerous are the antibiotic-resistant microorganisms, which, together with the bioproduction, can enter to the human and animal organisms from the modern agroecosystems (see Table II).

TABLE II: MICROORGANISMS RESISTANT TO TESTED ANTIBIOTICS ISOLATED FROM SOIL OF MODEL ECOSYSTEMS

Agroecosystem	Antibiotic resistance microorganisms (ARM)	Total number of ARM
Capsicum annum	<i>Enterococcus faecalis</i> , <i>Yersinia enterocolitica</i> , <i>Bacillus cereus</i> , <i>Bacillus megaterium</i>	4
Vitis vinifera	<i>Clostridium perfringens</i> , <i>Clostridium difficile</i> , <i>Escherichia coli</i> , <i>Enterococcus faecalis</i> , <i>Bacillus megaterium</i> , <i>Bacillus subtilis</i> , <i>Bacillus cereus</i> , <i>Bacillus licheniformis</i> , <i>Enterobacter cloacae</i> , <i>Serratia fonticola</i> , <i>Pantoea agglomerans</i> , <i>Hafnia Alvei</i>	12
Rubus idaeus. L	<i>Clostridium perfringens</i> , <i>Clostridium oedematiens</i> , <i>Clostridium difficile</i> , <i>Enterobacter cloacae</i> , <i>Enterococcus faecalis</i> , <i>Hafnia Alvei</i> , <i>Bacillus megaterium</i> , <i>Bacillus mycoides</i> , <i>Bacillus subtilis</i> , <i>Bacillus cereus</i> , <i>Escherichia coli</i>	11
Petroselinum crispum	<i>Bacillus megaterium</i> , <i>Bacillus licheniformis</i> , <i>Hafnia alvei</i> , <i>Bacillus cereus</i> , <i>Enterococcus faecalis</i>	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 of raspberry (*Rubus idaeus. L.*) were isolated following antibiotic resistance anaerobic microorganisms: *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 [17]. 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 [18]. 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*. All of them are antibiotic resistant and are the causative agents of foodborne 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.

IV. CONCLUSION

Soil microbiome of plants: *Capsicum annum*, *Vitis vinifera*, *Rubus idaeus* L., *Petroselinum crispum* 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. In total from communities of dominant bacteria were isolated 23% of microorganisms characterized by high level of antibiotic resistance. All of them are pathogenic or conditionally pathogenic for human and can cause food borne diseases.

REFERENCES

- [1] Thiele-Bruhn, S. Pharmaceutical antibiotic compounds in soils—a review. *Journal of Plant Nutrition and Soil Science*, Vol.166, no 2, pp.145-167, 2003.
<https://doi.org/10.1002/jpln.200390023>
- [2] Martínez, J. L. Antibiotics and antibiotic resistance genes in natural environments. *Science*, Vol.321, pp. 365-367, 2008.
<https://doi.org/10.1126/science.1159483>
- [3] Kümmerer, K. Resistance in the environment. *Journal Antimicrob Chemother*, Vol. 54 (2), pp. 311–320, 2004.
<https://doi.org/10.1093/jac/dkh325>
- [4] Pruden, A., Pei, R., Storteboom, H., & Carlson, K. H. Antibiotic resistance genes as emerging contaminants: studies in northern Colorado. *Environmental Science & Technology*, Vol. 40, no 23, pp.7445-7450, 2006.
<https://doi.org/10.1021/es060413l>
- [5] Wright, G. D. The antibiotic resistome. *Expert opinion on drug discovery*, Vol. 5(8), pp. 779-788, 2010.
<https://doi.org/10.1517/17460441.2010.497535>
- [6] Lyudmyla Symochko, Tamara Meleshko, Vitaliy Symochko, Nadiya Boyko. *International Journal of Ecosystems and Ecology Sciences*, Vol. 8, no 3, pp.591-598, 2018.
<https://doi.org/10.31407/ijees8320>
- [7] Boxall, A. B., Johnson, P., Smith, E. J., Sinclair, C. J., Stutt, E., & Levy, L. S. Uptake of veterinary medicines from soils into plants. *Journal of Agricultural and Food Chemistry*, Vol. 54, no 6, pp. 2288-2297, 2006.
<https://doi.org/10.1021/jf053041t>

- [8] Sannes M., Belongia E., Kieke B, Smith K. Predictors of antimicrobial-resistant *Escherichia coli* in the feces of vegetarians and newly hospitalized adults in Minnesota and Wisconsin. *J. Infect. Dis.*, Vol.197, pp.430–434, 2008.
<https://doi.org/10.1086/525530>
- [9] Zhang L, Kinkelaar D, Huang Y, Li Y, Li X, Wang H. Acquired antibiotic resistance: are we born with it? *Appl. Environ. Microbiol.*, Vol. 77:pp.7134–7144, 2011.
<https://doi.org/10.1128/AEM.05087-11>
- [10] Robinson C.J, Bohannon B.M, Young V.B. From structure to function: the ecology of host-associated microbial communities. *Microbiol. Mol. Biol., Rev.* 74, pp. 453–476, 2010.
<https://doi.org/10.1128/MMBR.00014-10>
- [11] Cavigelli, M. A., & Robertson, G. P. The functional significance of denitrifier community composition in a terrestrial ecosystem. *Ecology*, Vol.81, no 5, pp. 1402-1414, 2000.
[https://doi.org/10.1890/0012-9658\(2000\)081\[1402:TFSODC\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2000)081[1402:TFSODC]2.0.CO;2)
- [12] Hammesfahr U, Heuer H, Manzke B, Smalla K, Thiele-Bruhn S. Impact of the antibiotic sulfadiazine and pig manure on the microbial community structure in agricultural soils. *Soil Biol Biochem*, Vol.40, pp.1583–1591, 2008.
<https://doi.org/10.1016/j.soilbio.2008.01.010>
- [13] Heuer H, Smalla K. Manure and sulfadiazine synergistically increased bacterial antibiotic resistance in soil over at least two months. *Environ Microbiol*, Vol. 9, pp. 657–666, 2007.
<https://doi.org/10.1111/j.1462-2920.2006.01185.x>
- [14] Heuer H, Schmitt H, Smalla K. Antibiotic resistance gene spread due to manure application on agricultural fields. *Curr Opin Microbiol*, Vol.14, pp. 236–243, 2011
<https://doi.org/10.1016/j.mib.2011.04.009>
- [15] Torsvik V, Qvraås L. Microbial diversity and function in soil: from genes to ecosystems. *Curr Opin Microbiol*, Vol.5, pp. 240–245, 2002
[https://doi.org/10.1016/S1369-5274\(02\)00324-7](https://doi.org/10.1016/S1369-5274(02)00324-7)
- [16] Goldman, E., & Green, L. H. (Eds.). *Practical handbook of microbiology*, Third Edition. New York: Boca Raton, 2015, pp. 44-110.
- [17] Forsberg KJ, Reyes A, Wang B, Selleck EM, Sommer MOA, et al. The shared antibiotic resistome of soil bacteria and human pathogens. *Science*, Vol. 337, pp. 1107–1111, 2012.
<https://doi.org/10.1126/science.1220761>
- [18] Prospero J., Blades E, Mathison G, Naidu R. Interhemispheric transport of viable fungi and bacteria from Africa to the Caribbean with soil dust. *Aerobiologia*, Vol. 21, pp. 1–19, 2005.
<https://doi.org/10.1007/s10453-004-5872-7>



Lyudmyla Symochko was born in 24.06.1977 (Ukraine). Education: Master's degree of Ecology and Environment Protection (2000); Doctor's degree (PhD) by Specialty - 03.00.16 Ecology (2005); Associate Professor since 2008. Professional Career: Prof. Assoc., Dr., lecturer at the Faculty of Biology. Senior researcher at Scientific Research and Educational Centre of Molecular Microbiology and Mucosal Immunology, Uzhhorod National University, Ukraine. Symochko Lyudmyla – a specialist in environmental microbiology and soil ecology. Since 2008 she has focused on autecology and synecology researches of soil microbiota. Explores the soil resistome and the role of natural and transformed ecosystems as reserves of antibiotic-resistant microorganisms. She developing new and improving existing methods of bioindication and biotesting the edaphotops in different biogeocenosis. Held microbiological monitoring of soil in different types of ecosystems. Detects antibiotic-resistant opportunistic pathogens in the environment and provide they risk assessment to the human health. Author of over 120 scientific publications, incl. 55 in professional journals. Member of the editorial board of scientific journals: «American Journal of BioScience» (USA) www.sciencepublishinggroup.com; «Journal of Earth Science and Engineering» (USA) www.davidpublisher.org; «EC Microbiology» (USA) www.ecronicon.com/microbiology-articles.php; «International Journal of Ecosystems and Ecology Science» (USA) www.ijees.net/index.php.
Chairman of the Transcarpathian Section of the International Association of Universities Environmentalists (2013-present).