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Quantitative evaluation of bacterial groups involved in degradative processes and the nitrogen circuit in a soil cultivated with tomatoes

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Abstract The soil is a complex ecosystem, in which exist favorable interactions between the organisms that populate it, such as bacteria and actinomycetes, which have an important role in nutrient recycling and plant growth, plants being superior organisms that have a positive impact on microorganisms.

Several groups of bacteria (fulfilling various soil functions, degradative processes, synthesis of some antibiotics, transformation of nitrogen) which were isolated in the autumn season from a garden soil cultivated with tomatoes, have been studied in this paper.

Classical determinations were made according to standard methodology and focused on quantitative and qualitative microbial aspects.
The results we obtained revealed the role of the plant on the abundance of bacteria in the analyzed soil. Mesophilic aerobic bacteria, actinomycetes, nitrogen-fixing bacteria, aerobic and anaerobic, included in the genus *Azotobacter* and *Clostridium,* but also nitrifying bacteria dominated in soil samples under the influence of tomato plants, compared to the control soil.

Key words

nitrogen-fixing bacteria, *Azotobacter, Clostridium,* actinomycetes, soil

The soil is the habitat of a heterogeneous bacterial microflora, which fulfills various functions and contributes to a greater or lesser extent to increasing or maintaining soil fertility [3, 13].

Soil microbiota has an essential role in recycling nutrients in nature, plant growth and health [11, 20]. Bacteria dominate quantitatively compared to other microbial groups, being followed by actinomycetes and fungi. The number of microorganisms decreases with the depth of the soil [12]. According to the same authors, tomatoes quantitatively influence the soil microbiota,especially in the case of a prolonged monoculture that sensitizes bacteria and leads to an increase in the number of fungi at the expense of other microorganisms. Besides the plant, the microorganisms were influenced by the depth, but also by the interaction between these factors.

Nitrogen is an essential chemical for the growth of microorganisms and plants. Nitrogen reaches the ground through natural and anthropic pathways. One of the natural ways is the biological fixation of nitrogen [18]. Bacteria involved in nitrogen fixation are symbiotic, aerobic and anaerobic,both groups possess a catalytic enzyme called nitrogenase. These bacteria are involved in the conversion of molecular nitrogen to ammonia, which is easily assimilated by plants. On the other hand, nitrifying bacteria in the soil can turn ammonium into nitrites ($NO2^-$) and nitrates ($NO3^-$) [23] which can be used by plants [1]. These sources of nitrogen are included in the protein of living creatures, which is transferred along the food chain and recycled after their death [24].

Aerobic nitrogen-fixing bacteria are included in several genres, of which *Azotobacter* is the most widespread. Fixing bacteria living in anaerobic conditions are included in the genus *Clostridium* [14]. Both groups are bacylar, heterotrophic, and their contribution to the nitrogen fixation process is considered insignificant, as they compete for energy sources with other microorganisms in the soil. However, studies conducted by [19] shows that the annual nitrogen intake, fixed by free bacteria, amounts to 20 kg/ha, and the number recorded by them in a soil evaluated by was between 100-1000 UFC/g sol [15].

Oda et al (2014), have followed the influence of biologically fixed molecular nitrogen, with the condition of a high content of organic substance in the C: N ratio, and have noticed a number of benefits that this has on the profile of a soil, the production and the remediation of soil degraded [9].

This study examined the influence of tomato plants on microbial groups that perform important soil functions such as participation in the degradative processes, the biological fixation of the molecular nitrogen and the nitrification processes.

Material and Methods

The garden soil we studied is located in the Western part of Romania. The harvest of samples happened in October, from the depth of 0-20 cm, from a parcel with tomatoes. The soil samples were brought to the Microbiology Laboratory within the University of Agricultural Sciences and Veterinary Medicine “King Michael I of Romania” from Timişoara, where they were mixed in a medium sample, which was prepared for microbiological analysis. In parallel, a control sample, not influenced by cultivated vegetables, was also studied. The incubation temperature of the isolated microorganisms was 28^{o}C, and the time was variable depending on the bacterial group.

The main microbiological determinations [**17**] were:

- Quantitative estimation of mesophilic aerobic bacteria (UFC/g sol), done by the streak plate method on nutrient agar medium;
- Quantitative evaluation of actinomycetes (UFC/g sol), done by the streak plate method, on Gause medium;
- Determining the probable number of free nitrogen-fixing bacteria, done by the serial dilution method, on mineral medium Ashby;
- Determining the probable number of aerobic nitrogen-fixing bacteria of the genus *Azotobacter*;
- Determining the probable number of aerobic nitrogen-fixing bacteria of the genus *Clostridium*;
- Determining the probable number of nitrifying bacteria, done by the streak plate method, on mineral ammonium nitrate based medium.

Results and Discussions

The soil is a complex, dynamic system, considered a determinant of the microbial community in the area of plant influence, which has a significant effect on microbial taxonomy [4]. The results obtained by microbial soil analysis are highlighted in the figures 1-3.

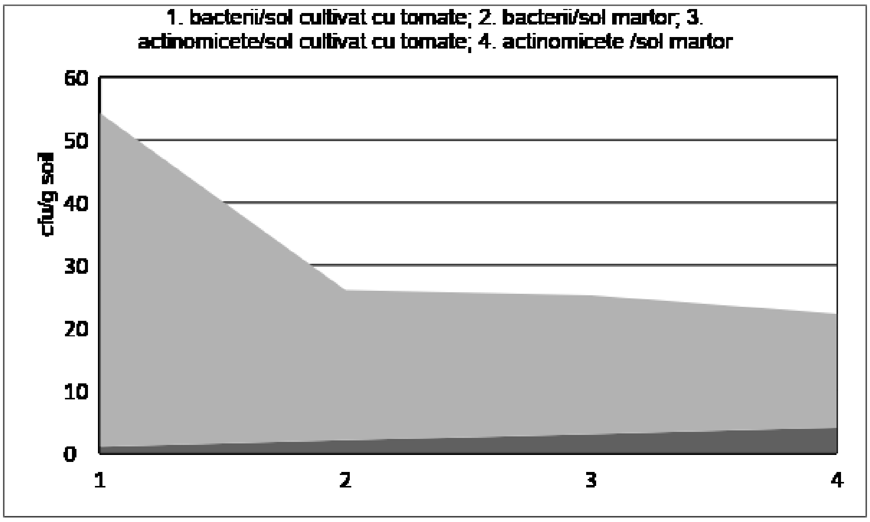

Fig. 1. Evolution of mesophilic aerobic bacteria and actinomycetes (UFC/g sol)

After a 48 hour incubation period (for bacteria), respectively 5 days (for actinomycetes), there was an increase in the number of bacteria and actinomycetes compared to the control sample (fig. 1).

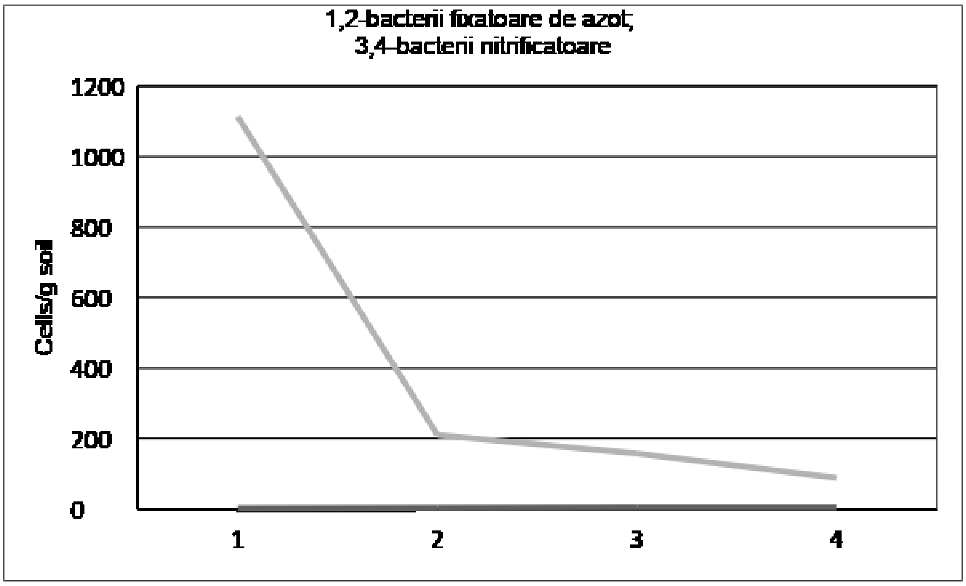

Fig. 2. Probable number of free nitrogen-fixing and nitrifying bacteria (cells/g soil)
Legend: 1.Total nitrogen-fixing bacteria from tomatoes influenced soil; 2. Total nitrogen-fixing bacteria from the control soil; 3. Nitrifying bacteria from tomatoes influenced soil; 4. Nitrifying bacteria from control soil

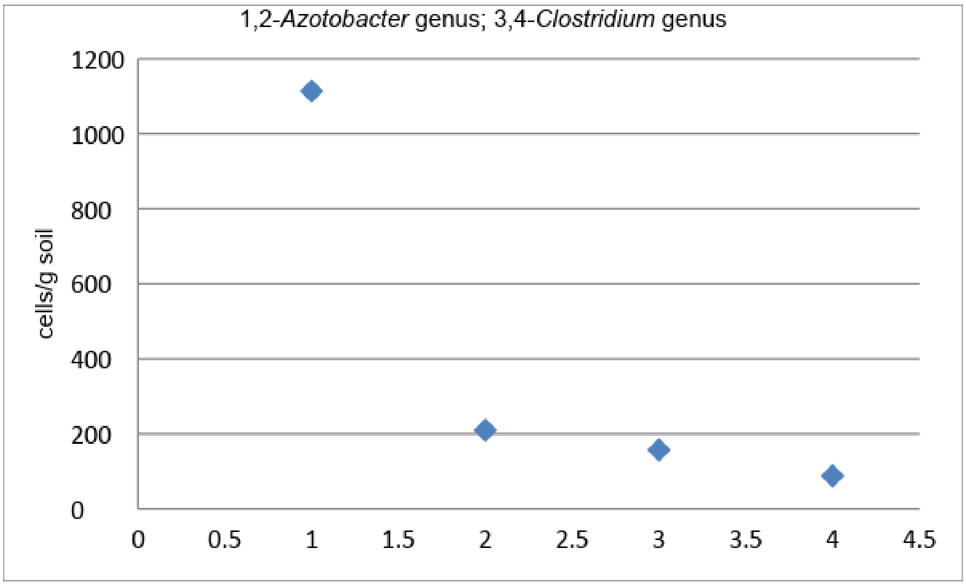

Fig. 3. Probable number of nitrogen-fixing bacteria in genres *Azotobacter* and *Clostridium*
Legenda: 1. Genus *Azotobacter* /soil with cultivated tomatoes; 2. Genus *Azotobacter* / control soil; 3. Genus *Clostridium* /soil with cultivated tomatoes; 4. Genul *Clostridium* / control soil.

Nitrogen-fixing bacteria were found after a week of incubation, and the nitrifying ones after 3 weeks. The differences between the soil samples influenced by the plant and the control one are obvious in the case of nitrogen-fixing bacteria and less noticeable for nitrifying bacteria (fig. 2). If we refer to the main types of nitrogen-fixing bacteria, we notice a great quantity of *Azotobacter,* which includes aerobic fixers, to the detriment of the genus *Clostridium,* which includes anaerobic fixers(fig. 3).

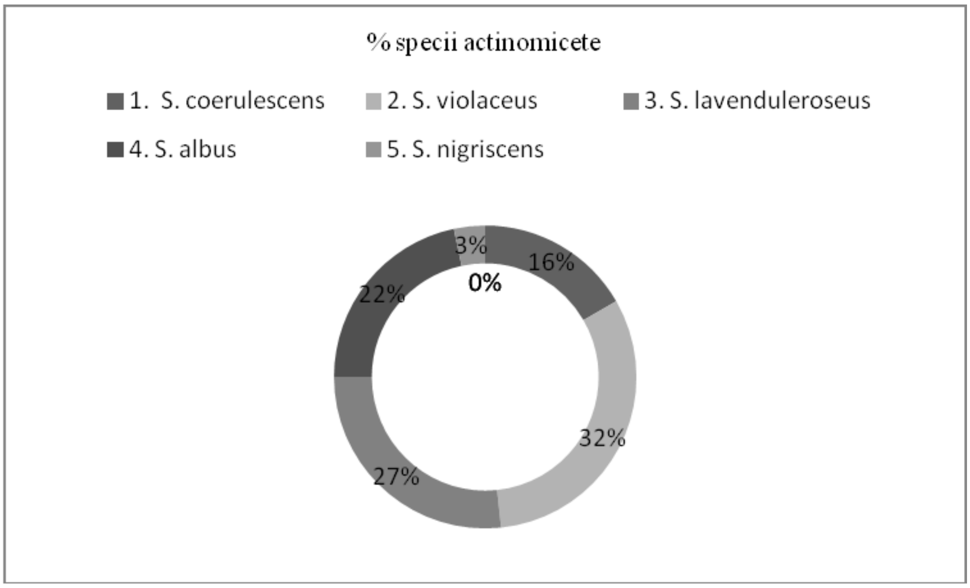

Fig. 4 Species of the genus *Streptomyces*

Species of actinomycetes, present in the analyzed soil are quantitatively successive, in the following order *S. violaceus>S. lavenduleroseus>S. albus>S. coerulescens>S. nigrescens.* The dominant species is *S. violaceus*, and the species present in the lowest number is *S. nigrescens.*

The microbial community in the plant-affected area is more abundant than the one existing in the control soil, fact mentioned by [21]. According to some studies, an important role in the bacterial groups is also the pH of the soil [5, 7] which in this case ranges from weak acid to neutral. To this factor we can also add the climate [10, 22], the type of soil [2, 6] and the availability of nutrients [8]. Also, in the studies conducted by Sun and collaborators (2017), a very rich bacterial flora was discovered in October and September, as evidenced by the studies in this paper [16].

Conclusions

There is a close relationship between microorganisms-plants-soil, with positive influences on microorganisms, fact observed by the analysis of the samples we studied in this paper.

Thus, the bacterial community in the soil samples analyzed is more abundant under the presence of tomato plants, compared to the control one. The number of bacteria from the genus *Azotobacter* numerically exceed the bacterial cells belonging to the anaerobic genus, *Clostridium.*

The composition of actinomycetes, which are among the microorganisms with an important role in the decomposition of hardly biodegradable substances and the synthesis of some antibiotics, is reduced to a few dominant species (*S. violaceus, S. lavenduleroseus* și *S. albus*).

References

1.Bernhard A., 2010, The Nitrogen Cycle: Processes, Players, and Human Impact. Nature Education Knowledge 3(10):25.

2.Girvan M.S., Bullimore J., Pretty J.N., Osborn A.M., Ball A.S., 2003, Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils. Appl Environ Microbiol. 69: 1800–1809.

3.Hossein R., Eskash A., Shariatmadari Z., 2011, Effect of bacterial and cyanobacterial culture on growth, quality and yield of *Agaricus bisporus*, Proceedings of the 7th International Conference on Mushroom Biology and Mushroom Products (ICMBMP7), 2011, Section: Waste conversion, substrates and casing: 406-411.

4.Jacoby R., Peukert M., Succurro A., Koprivova A., Kopriva S., 2017, The Role of Soil Microorganisms in Plant Mineral Nutrition—Current Knowledge and Future Directions. Frontiers in Plant Science, vol. 8: 1-19.

5.Kim J.M., Roh A-S, Choi S-C, Kim E-J, Choi M-T, Ahn B.K., et al., 2016, Soil pH and electrical conductivity are key edaphic factors shaping bacterial communities of greenhouse soils in Korea. J Microbiol., 54: 838–845.

6.Lauber C.L., Hamady M., Knight R., Fierer N., 2009, Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Appl. Environ. Microbiol., 75: 5111–5120.

7.Lauber C.L., Strickland M.S., Bradford M.A., Fierer N., 2008, The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biol Biochem. 40: 2407–2415.
8.Leff J.W., Jones S.E., Prober S.M., Barberán A., Borer E.T., et al., 2015, Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. Proc Natl Acad Sci. 112: 10967–10972.
9.Oda Masato, Kenji Tamura, Hiroko Nakatsuka, Miki Nakata, Yukimi Hayashi, 2014, Application of High Carbon: Nitrogen Material Enhanced the Formation of the Soil A Horizon and Nitrogen Fixation in a Tropical Agricultural Field. Agricultural Sciences, 2014, 5, 1172-1181.
10.Maestre F.T., Delgado-Baquerizo M., Jeffries T.C., Eldridge D.J., Ochoa V., Beatriz G., et al., 2015, Increasing aridity reduces soil microbial diversity and abundance in global drylands. Proc Natl Acad Sci. 112: 15684–15689.
11.Maul J.E., Buyer J.S., Lehmanb M.R., Culmanc S., Blackwood C.B., Roberts D.P., Zasada I.A., Teasdalea J.R., 2014, Microbial community structure and abundance in the rhizosphere and bulk soil of a tomato cropping system that includes cover crops, Applied Soil Ecology, 77: 42–50.
12.Mo A.S., Qiu Z.Q., He Q., Wu H.Y., Zhou X.B., 2016, Effect of Continuous Monocropping of Tomato on Soil Microorganism and Microbial Biomass Carbon. Journal Communications in Soil Science and Plant Analysis, vol. 47, Issue 9: 1069-1077.
13.Pace N.R., 1997. A molecular view of microbial diversity and the biosphere. Science 276: 734–740.
14.Postgate, J. R., 1982, The Fundamentals of Nitrogen Fixation. New York, NY: Cambridge University Press.
15.Šimon T., 2003, Utilization of the biological nitrogen fixation for soil evaluation. Plant soil environ., 49 (8): 359–363.
16.Sun S., Li S., Avera B.N., Strahm B.D., Badgleya B.D., 2017, Soil Bacterial and Fungal Communities Show Distinct Recovery Patterns during Forest Ecosystem Restoration. Applied and Environmental Microbiology, Vol. 83, Issue 14: 1-14.
17.Stefanic, G., Irimescu Orzan M.E., Gheorghita N., 2001, The possibility to estimate the level of soil fertility by modular and synthetic indices. Romanian Agricultural Research, No. 59-70.
18.Vance, C., 2001, Symbiotic nitrogen fixation and phosphorus acquisition. Plant nutrition in a world of declining renewable resouces. Plant Physiology 127: 391-397.
19.Vadakattu, G., Paterson, J. 2006. Free-living bacteria lift soil nitrogen supply. Farming Ahead 169, 40.
20.Wang R., Zhang H., Sun L., Qi G., Chen S., Zhao X., 2017, Microbial community composition is related to soil biological and chemical properties and bacterial wilt outbreak, Sci. Rep., 7: 343:1-10.
21.Wang B., Adachi Y., Sugiyama S., 2018. Soil productivity and structure of bacterial and fungal communities in unfertilized arable soil, Plos One, https://doi.org/10.1371/journal.pone.0204085
22.Zhou J., Deng Y., Shen L., Wen C., Yan Q., Ning D., et al. 2016. Temperature mediates continental-scale diversity of microbes in forest soils. Nat Commun. 7: 12083.
23.https://www.visionlearning.com/en/library/Earth-Science/6/The-Nitrogen-Cycle/98
24.https://www.lenntech.com/nitrogen-cycle.htm