THE CELLULOLYTIC MICROORGANISMS AND THE FUNGI IN MOUNTAIN PASTURES DOMINATED BY *AGROSTIS TENUIS* L

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Abstract: *Our research was focused on the activity of cellulolytic microorganisms, but we also highlighted the main species involved in this process, such as the fungi in the soils of spontaneous mountain pastures in 14 locations from Caras-Severin County. The main locations are: Zlatita (C1), Oravita (C2), Anina (C3), Valiug (C4), Bigar (C5), Cuptoare (C6), Garana (C7), Semenic Piatra Goznei (C8), Semenic Station (C9), Barbosu (C10), Eftimie Murgu (C11), Valea Pai (C12), Carasova (C13) and Carasova livada (C14). The dominant species of gramineous plants in these grasslands was A. tenuis L. The experiments took place in laboratory conditions. The isolation of the species and genera of bacteria and fungi was performed on specific growth media for each microbial group, respectively. According to the results obtained, the activity of cellulolytic microorganisms in the tested soils (the range of microbial species involved and the genera of fungi) is generally limited.*

Key words: *cellulolytic activity, mountain area, A. tenuis L., fungi, cellulolytic bacteria.*

INTRODUCTION

Agrostis tenuis (*A. tenuis*), known as colonial bent, common bent or brown top, belongs to the family *Poaceae* or *Gramineae*, genus *Agrostis*. It is one of the main plants in pastures and hay meadows, in moderately moist areas on hills and mountains. It can be found at altitudes of over 300 m, up to 1200 m (MARUȘCA et al., 2010).

From a morphological and biological point of view, it appears as rare bushes of approximately 40 to 60 cm, with short rhizomes and complex inflorescence (panicle). It prefers acid soils that are poor in nutrients, but it can also be found on neutral soils. This species of gramineous plants, valuable as fodder, is either dominant or co-dominant, having a medium to superior ecological quality. The quantity of aftergrass is relatively good, the species having medium productivity (MARUȘCA ET AL., 2010; EDGAR AND CONNOR, 1991, 2000; DECISION 78/2015; SĂNDOIU I. et al., 2014,)

A group of researchers from Ukraine noticed small individual differences among the *A. tenuis* plants from different countries, and they put together a wide genetic base that will be used in improvement processes for obtaining new varieties suitable for lawns or other recreational setups (STUKONIS VACLOVAS AND SLEPETYS JONAS/site).

The studies published by HUMPHREYS and NICHOLL (1984) and WATKINS and MACNAIR (1991) addressed the tolerance of *A.tenuis* L to metals and highlight how important the genetic background of the plant is for the plant's tolerance to metals. Besides, SUDOVÁET et al. (2008) reported better growth of species *A. tenuis* in fungal associations, where the soil is contaminated by metals.

Research has shown that in resown soils covered by pastures the C/N ratio is big, the quantity of available nutrients is small and the microbial activity is limited. Application of sodium nitrate and phosphate stimulates cellulolytic activity (KIRKWOOD R. C., 1964).

The evaluation of the biochemical properties of the soils under pastures proved that there are no big differences among the data obtained in different countries (PAZ-FERREIRO, 2007).

Among the factors that can influence the biochemical properties of the soil, PAZ-FERREIRO et al. (2011) mention the area where the soil is located, the temperature, humidity and soil management.

Taking into account the importance of the cellulolytic process in the circuit of C, our study presents the microbial species involved in the cellulolytic processes and the fungi in the soil samples taken from mountain pastures in Caras-Severin County.

MATERIAL AND METHODS

The soil samples we tested were taken from pastures found at different altitudes in Banat Mountains, Caras-Severin County. The locations are as follows: Zlatita (**C1),** Oravita (**C2),** Anina (**C3),** Valiug (**C4),** Bigar (**C5),** Cuptoare **(C6),** Garana (**C7),** Semenic Piatra Goznei (**C8),** Semenic Station (**C9),** Barbosu (**C10),** Eftimie Murgu (**C11),** Valea Pai (**C12),** Carasova (**C13)** and Carasova livada (**C14).** *A. tenuis L* was the dominant gramineous plant in the pastures in all 14 locations. The humidity and reaction of the soil samples were determined when the samples were collected.

The soil samples were taken from 0-10 cm depth. The processing, the conditioning and the experiment set-up were made in the Microbiology laboratory within BUASVM Timisoara. The principle of the method used for determining the cellulolytic activity was that of Stefanic Gh., (2006). Sample incubation period was 3 weeks and the optimal growth period was 28^{o}C. The isolation and identification of the microorganisms involved in cellulolysis were performed on solid Stapp medium, where the source of C is the cellulose in the filter paper. The incubation lasted for 10 days, at the same temperature.

After the rate of microorganism evolution in the soil was established (the data were presented in a previous paper), the genera of fungi present in these soil samples were identified (Borozan, 2006). The medium for the fungi was Sabouraud-chloramphenicol. The optimal growth temperature was 28^{o}C, for 5 days. The molds were identified based on their microscopic and macroscopic characteristics.

RESULTS AND DISCUSSIONS

The cellulolytic and fungal microorganisms found in the soil samples from the 14 locations are represented in Figures 1 and 2.

The cellulose degrading capacity in the soils of mountain pastures was generally limited. The highest cellulolytic activity was found in soils from locations C1 and C9, while the lowest activity of cellulolytic microorganisms was present in areas C12, C8, C7 and C13.

The proportion between the two groups of cellulolytic microbes (bacteria and fungi) varies. In some areas, either a certain bacterial species or a fungus species is dominant (Z6-13). Of the bacterial species involved in the cellulolytic process, we isolated the following: *Cellvibrio flavescens*, *Cellfalcicola viridis, Cellfalcicula mucosa*, *Cellvibrioochracea*, *Cytophagarubra and Cellfalciculafusca*. No fungi withcellulolyticactivity were identified. The highest percentage obtained by fungi was registered in area Z3, and the highest percentage obtained by bacteria was in area Z1 (Fig. 1).

Paz-Ferreiro et al. (2010) noted that altitude influences the climatic conditions, which in turn has an effect on the microorganisms that are involved in the processes in the soil, such as cellulolytic activity, proteolytic activity and nitrogen mineralization.

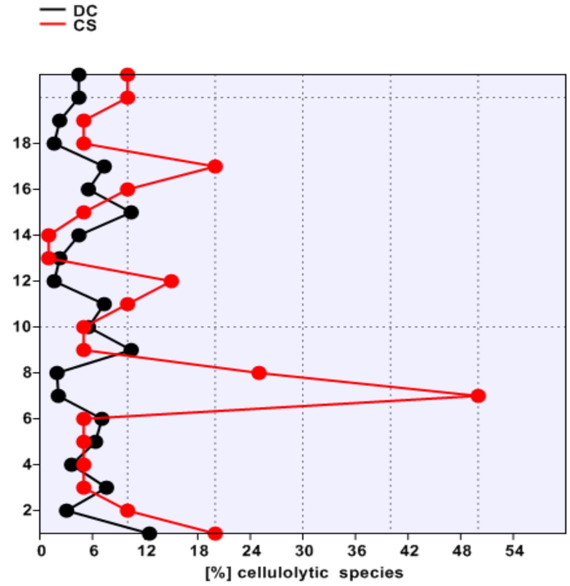

Fig. 1. The cellulolytic activity of the species of microorganisms in the tested soils
Legend: DC = % degraded cellulose; CS = % cellulolytic species

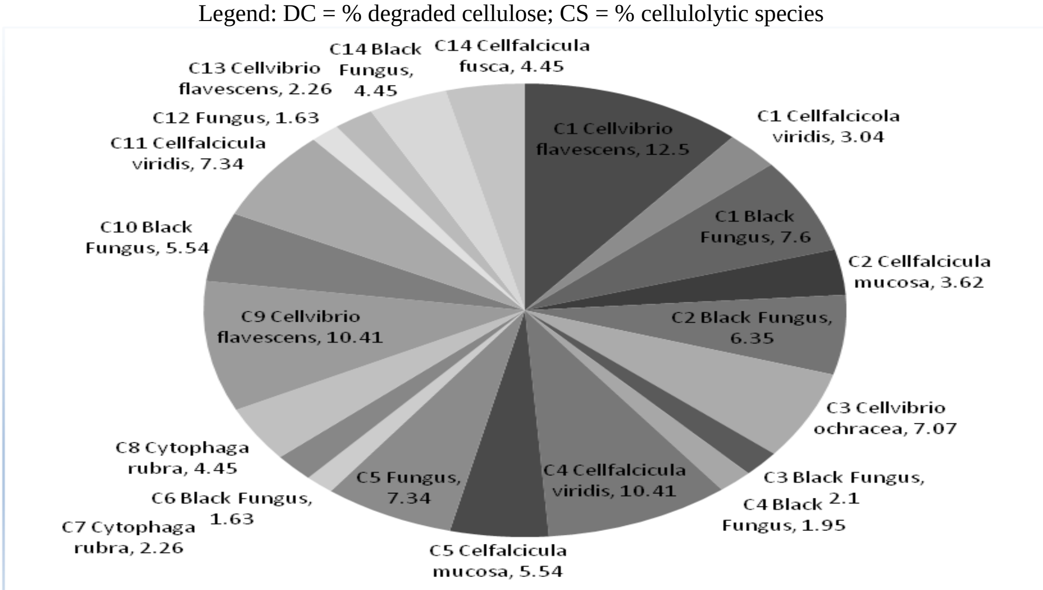

Fig. 2. The fungal genera isolated from the tested soil samples

Of the filamentous fungi isolated off the soil granules, the following appeared in higher percentage: *Monosporium (C8), Aspergillus (C13, C9), Penicillium* (C3), *Humicola* (C6, C9), *Acremonium* (C6,C7,C9) and Mucor (C1,C4), (Fig. 2).

The way in which the soil is exploited has an influence on the structure of the microbial community, leading to imbalance which can in turn bring about significant reductions of the fungal biomass in the soil, compared to the bacterial load, and changes in the cycle of chemical elements. These changes seem to be linked to plant productivity and composition, but also to the type and quantity of fertilizers applied (DONNISON et al., 2000).

CONCLUSIONS

In pastures covered mostly by *A. tenuis* L, the cellulolytic activity and the variation of bacterium and fungus species involved in this process are low. Altitude, abiotic factors, plants and soil management bring an important contribution to the appearance of the inhibiting or stimulating effect of the microbial activity in the soil. Of the filamentous fungi isolated in the soil samples, higher percentages were found from genera *Monosporium, Aspergillus, Penicillium*, *Humicola*, *Acremonium* and *Mucor.*

BIBLIOGRAPHY

1. BOROZAN BREICA AURICA, 2006. Microbiologie (îndrumător de lucrări practice), Editura Mirton, Timişoara.
2. EDGAR E, CONNOR H., Flora of New Zealand, vol. Gramineae, Manaaki Whenua Press, 2000
3. EDGAR, E.; FORDE, M. B. 1991. *Agrostis* in New Zealand. *New Zealand Journal of Botany 29*: 139–161.
4. DONNISON M. LOUISE, GRIFFITH S.GWYN, HEDGER JOHN, HOBBS J.PHIL, BARDGETT D.RICHARD, 2000. Management influences on soil microbial communities and their function in botanically diverse hay meadows of northern England and Wales, Soil Biology and Biochemistry, Volume 32, Issue 2, pp 253–263.
5. HUMPHREYS M. O., NICHOLL M. K., 1984. Relationships between tolerance to heavy metals in *Agrostis capillaries* L. (*A. Tenuis Sibth.*), New Phytologist, Volume 98, Issue 1, pp 177–190.
6. KIRKWOOD R. C., 1964, A study of some factors causing mat formation of reseeded hill pasture swards, Grass and Forage Science, Volume 19, Issue 4, pp. 387–395.
7. MARUŞCA TEODOR, MOCANU VASILE, CARDAŞOL VASILE, HERMENEAN IOAN, BLAJ VASILE ADRIAN, OPREA GEORGETA, TOD MONICA ALEXANDRINA, 2010. Ghid de producere ecologică a furajelor de pajisti montane, Editura Universitătii Transilvania din Brasov.
8. PAZ-FERREIRO J., TRASAR-CEPEDA C., LEIRÓS M. C., S. SEOANE, F. GIL-SOTRES 2007. Biochemical properties of acid soils under native grassland in a temperate humid zone, New Zealand Journal of Agricultural Research, Vol. 50: 537-548.
9. PAZ-FERREIRO JORGE, TRASAR-CEPEDA CARMEN, LEIRÓS CARMEN M., SEOANE SOCORRO, GIL-SOTRES, 2010. Effect of management and climate on biochemical properties of grassland soils from Galicia (NW Spain), Volume 46, Issue 2, pp. 136–143.
10. PAZ-FERREIRO JORGE, TRASAR-CEPEDA CARMEN, LEIRÓS MARIA DEL CARMEN, SEOANE SOCORRO, GIL-SOTRES FERNANDO, 2011. Intra-annual variation in biochemical properties and the biochemical equilibrium of different grassland soils under contrasting management and climate, Biology and Fertility of Soils, Volume 47, Issue 6, pp 633-645.
11. SĂNDOIU I., COJOCARIU LUMINIŢA**,** REY R., CALUŞERU LAVINIA ALINA, ILIE R., HORABLAGA M., 2014, The distribution of species *Agrostis tenuis* L. across the grasslands in Banat Mountains, Romanian Journal of Grassland and Forage Crops, No.10/2014, p: 67-74, Cluj Napoca, Romania.

12. SUDOVÁ RADKA, PAVLA DOUBKOVÁ, MIROSLAV VOSÁTKA, 2008. Mycorrhizal association of *Agrostis capillaries* and *Glomusin traradices* under heavy metal stress: Combination of plant clones and fungal isolates from contaminated and uncontaminated substrates, Applied Soil Ecology, Volume 40, Issue 1, pp. 19–29.
13. STUKONIS VACLOVAS, SLEPETYS JONAS, Assessment of *Agrostis capillaris* wild populations for use in turf grass breeding (http://www.lzi.lt/projektai/C3_C4_zol_augal_files/PRIEKULE_%20posteris_2013.pdf)
14. STEFANIC GH., 2006. Methods of soil analysis (biological, chemical and enzymatic), II[th] Edition, ICDA Fundulea, XXVIII.
15. WATKINS A J AND MACNAIR M R, 1991. Genetics of arsenic tolerance in *Agrostis capillaries* L., Heredity, 66, 47–54; doi:10.1038/hdy.1991.6
16. DECISION NO. 78/2015 regarding the amending and supplementing Methodological Norms for the application of Government Emergency Ordinance no. 34/2013 on the organization, management and operation of permanent grassland and land amending and supplementing Law no. 18/1991, approved by Government Decision no. 1064/2013.