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# MICROBIOLOGICAL CHARACTERIZATION OF SUPPRESSIVE FOREST SOIL FROM ENISALA

***MATEI GABI-MIRELA, *MATEI S., MOCANU VICTORIA, DUMITRU SORINA***
*National Research-Development Institute for Soil Science, Agrochemistry and Environment-Bucharest, Bd. Mărăşti 61, 011464, Sector 1, Bucharest, Romania;*
**e-mail:so_matei602003@yahoo.com*

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## *ABSTRACT*

*Suppressiveness is the property of certain soils to inhibit or limit the development of pathogenic species due to the presence of microbial antagonists.*

*Research has been carried out to characterize the microbial communities in the Calcic Chernozem (WRB) under cultivated forest from Enisala, Tulcea county. The bacterial microflora was dominated by pseudomonads and actinomycetes and fungal species were cosmopolitan, many of them antagonists and strong cellulolytic (Trichoderma, Mortierella, Epicoccum, Aspergillus, Penicillium). Soil suppressiveness was confirmed by the absence of the soil-borne pathogenic species. Mechanisms such as biochemical antagonism and hiperparasitism were evidenced by examination of interaction zone between Trichoderma viride and the test-pathogen Fusarium verticillioides.*

## INTRODUCTION

The presence of antagonistic microorganisms is the source of soil suppressiveness because of their capacity to respond sensitively to environmental stress that can favour the increasing of pathogen effectives (Alabouvette, 1986; Alabouvette and Steinberg, 2006).

Among microbial species able to act as efficient agents for the biocontrol of soil-borne plant pathogens literature cites certain representatives of bacterial genera *Pseudomonas* (Attitala et al., 2001; Mitoi et al., 2012), *Bacillus* (Sicuia et al., 2013), various lactic acid bacteria (Magnusson et al., 2003), actinomycetes and fungal genera *Trichoderma, Gliocladium, Epicoccum, Trichotecium,* non-pathogenic *Fusarium* (Cornea et al., 2009; Shahid et al., 2013; Matei et al., 2014).

Research has been carried out to characterize the microbial communities in the Calcic Chernozem (WRB) under cultivated forest from Enisala, Tulcea county and to evidence the microorganisms and mechanisms responsible for suppressiveness,

## MATERIAL AND METHOD

Microbiological parameters such as: total counts of aerobic heterotrophic bacteria (on Topping medium), total counts of fungi (on PDA) were estimated by soil dillution method, and repported to 1g dry soil.

Stapp culture medium overlayed with filter paper was used for cultivation of cellulolytic microorganisms (Papacostea, 1976).

Taxonomic identification of microorganisms developed on culture media after incubation in the dark at 25$^{o}$C was done according to determinative manuals of Bergey (1994), Watanabe (2002), Domsch şi Gams (1970).

The global physiological activities of microflora were determined by substrate induced respiration method (SIR) and expressed as mg $CO_2$x100g$^{-1}$ soil (Matei, 2011).

The presence of antagonistic microorganisms responsible for soil suppressiveness was evidenced by overlay assay method (Postma et al., 2008) and dual cultures (Phuoc, 1988).

Micrographs were carried out to illustrate the aspects of hiperparasitism or chemical antagonism revealed by examination of interaction zones at optic microscope.

## RESULTS AND DISCUSSIONS

Calcic chernozem from Enisala under cultivated forest has very good physical properties, determined by loamy texture, satisfactory structure, low values of bulk density (1,27g/cm$^3$), high porosity (53-55%), good permeability and moderate available moisture holding capacity, with pH values over 6,87.

Soil presents very high values of microbiological parameters in Am1 (0-10cm) de surface horizon, with one order of magnitude superior to the values registered for the rest of horizons (Table 1).

*Table 1*

**Microbial counts and potential soil respiration in Calcic Chernozem from Enisala**

| **Horizon/ Depth (cm)** | **Fungal counts** *x 10$^3$cfu x g$^{-1}$ dry soil* | **Bacterial counts** *x 10$^6$ viable cells x g$^{-1}$ dry soil* | **Soil respiration** *mg $CO_2$x100 g$^{-1}$ soil* |
|---|---|---|---|
| **Am1 (0-10)** | 108,738 | 696,313 | 158,505 |
| **Am2 (10-25)** | 47,514 | 16,876 | 34,034 |
| **Am3 (25-50)** | 65,477 | 18,010 | 50,785 |
| **A/C (50-63)** | 52,380 | 3,755 | 28,732 |

Thus, bacteria are present with moderate effectives in Am2, Am3 horizons and low in A/C. Values estimated for fungi show a moderate density .

The level of potential soil respiration reflects numeric distribution of the main groups of microorganisms and presents moderate intensity in Am2, Am3 horizons and a low level in transition horizon A/C.

From the taxonomic point of view, in Am1 (0-10cm) surface horizon are present associations of genera *Pseudomonas* (fluorescent and non-fluorescent) with bacillaceae, *Arthrobacter* and white Actinomycetes (Table 2).

In Am2 horizon are present actinomycetes from Series Albus and Fuscus, representatives of genera *Pseudomonas, Bacillus* and few species less encountered in soils, *Serratia marcescens* and *Mycobacterium roseum*, that develop red colonies.

In Am3 horizon is dominant *Bacillus megaterium* accompanied by species of *Pseudomonas* and actinomycetes from Series Fuscus, Albus and Ruber. In A/C horizon were identified species of *Pseudomonas, Bacillus* and Actinomycetes producing brown pigments.

In Petri plates with PDA culture medium plated with soil dilutions from surface Am1 horizon (0-10cm) developed fungi belonging to antagonistic species *Paecilomyces marquandii*, with abundant growth, covering an important area of the plate surface in 14 days.

An association is remarked between species *Mortierella minutissima-Penicillium nigricans,* along with cellulolytic *Chaetomium globosum*, Actinomycetes, *Epicoccum nigrum* and bacteria *Cytophaga* sp.

*Table 2*

**Taxonomic composition of bacterial and fungal microflora in Calcic Chernozem from Enisala**

| **Horizon/ Depth** (cm) | **Bacterial species** (Topping) | **Fungal species/cellulolytic microflora** (PDA/Stapp) |
|---|---|---|
| **Am1 (0-10)** | *Pseudomonas fluorescens,* *Bacillus subtilis* *Pseudomonas* sp., *Bacillus circulans,* *Bacillus cereus* var. *mycoides,* *Arthrobacter citreus* Actinomycetes Series Albus | *Paecilomyces marquandii,* *Mortierella* sp., *Penicillium nigricans,* *Geotrichum candidum/* Actinomycetes Series Griseus *Trichoderma viride,* *Mortierella minutissima,* *Chaetomium globosum,* *Mucor racemosus,* *Epicoccum nigrum,* *Humicola grisea,* *Cytophaga* sp. |
| **Am2 (10-25)** | *Pseudomonas* sp., *Bacillus cereus,* *Serratia marcescens* *Mycobacterium roseum,* Actinomycetes Series Albus and Fuscus | *Mucor racemosus,* *Penicillium albidum,* *Penicillium verrucosum /* *Stachybotrys chartarum,* *Humicola grisea,* *Cunninghamella elegans,* *Trichoderma viride,* *Epicoccum nigrum,* *Phaeotrichosphaeria sp.,* *Mortierella minutissima,* *Cytophaga* sp. Actinomycetes Series Albus and Griseus |
| **Am3 (25-50)** | *Bacillus megaterium,* *Pseudomonas fluorescens,* *Pseudomonas* sp., *Pseudomonas pseudogleyi,* Actinomycetes Series Albus, Fuscus and Ruber | *Mortierella minutissima,* *Penicillium verrucosum,* *Penicillium aurantiogriseum,* *Epicoccum nigrum,* *Fusarium oxysporum /* *Myrothecium verrucaria* *Mortierella minutissima,* *Epicoccum nigrum,* *Penicillium glabrum* |
| **A/C (50-63)** | *Pseudomonas* sp., *Bacillus sphaericus,* Actinomycetes Series Fuscus | *Penicillium verrucosum,* *Phialophora fastigiata,* *Mortierella minutissima,* *Penicillium* sp., *Aspegillus terreus /* Actinomycetes Series Albus and Fuscus *Myrothecium verrucaria,* *Aspegillus sp.,* *Absidia corymbifera,* *Penicillium glabrum* |

*Mucor racemosus* is well developed in Am2 horizon (10-25cm) but in competition with *Penicillium* that overgrows it as white colonies (*Penicillium albidum*).

In this horizon, cellulolytic microflora is very well developed, dominated especially by black colonies of *Stachybotrys chartarum*, extremely efficient in degradation of cellulose, near representatives of genera *Cunninghamella*, *Trichoderma, Humicola, Epicoccum, Mortierella*, Ascomycetes from genera *Chaetomium* and *Phaetrichosphaeria*, as well as various actinomycetes or bacteria *Cytophaga* sp (Fig.1).

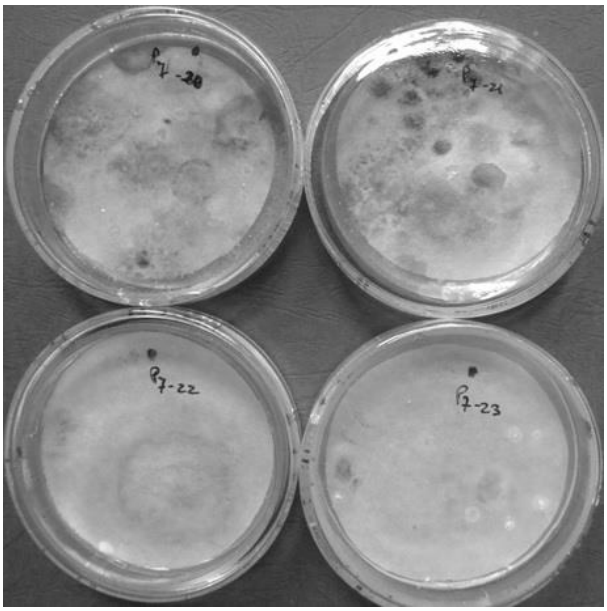


**Figure 1 Cellulolytic microflora in soil from Enisala - Stapp medium**

Associations of genera *Mortierella, Penicillium* and *Epicoccum* are characteristic for fungal communities from Am3 horizon. Another efficient cellulolytic fungus, *Myrothecium verrucarria* was identified on Stapp medium.

Two species from genus *Penicillium* are dominant in transition horizon A/C (50-63cm), followed by *Umbelopsis nana (Mortierella nana*) and *Phialophora fastigiata*.

In this horizont, also identified colonies of *Aspergillus terreus* with cellulolytic and antimicrobial capabilities, actinomycetes with large colonies, pale pink or in dark nuances.

Cellulolytic species *Penicillium glabrum* capable to release K from silicates in forms accessible for plants . Its activity is possibly correlated with the important quantity of available potassium in all horizons of soil profile.

The presence of good physical-chemical conditions, an active antagonistic microflora and the absence of soil-borne plant pathogens are arguments supporting the suppressive character of soil from Enisala.

In order to verify the microbial nature of suppressiveness, Petri plates with microflora from Am2 horizon (10-25cm) were overlayed with PDA medium inoculated with suspension of spores belonging to pathogen species *Fusarium verticillioides* and after 5 days observations have been carried out for identifying representatives of microbial population capable to create zones of growth inhibition in mycelial layer of pathogen.

Photographs showed the existence of zones of growth inhibition of pathogen *Fusarium verticillioides* around colonies of actinomycetes, due to their antagonistic activity against plant pathogen and evidenced their role in conferring the characteristic of suppressiveness to the soil (Fig. 2).

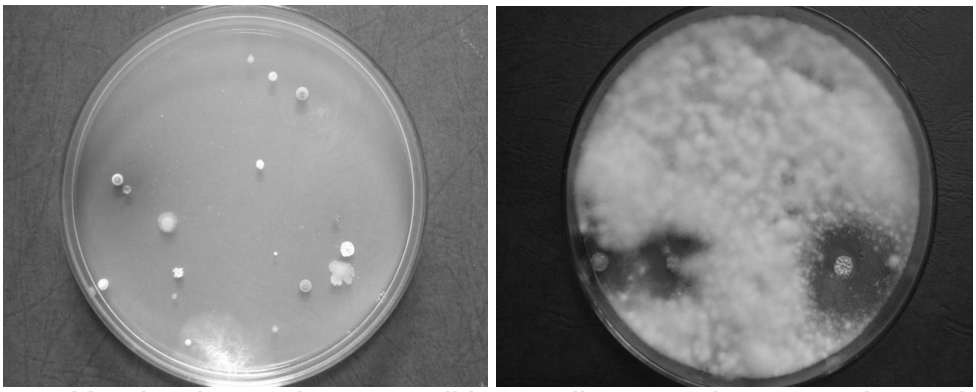

**Figure 2 Evidencing the species responsible for soil suppressiveness against pathogen- overlay assay**

The existence of 3-4 mm clear zones between the two microorganisms revealed that the antagonism was based on producing of secondary metabolites with inhibitory role. Hiperparasitism was another control mechanism involved, as showm in Fig.3, where actinomycete developed its structures around fungal hyphae of *Fusarium verticillioides.*

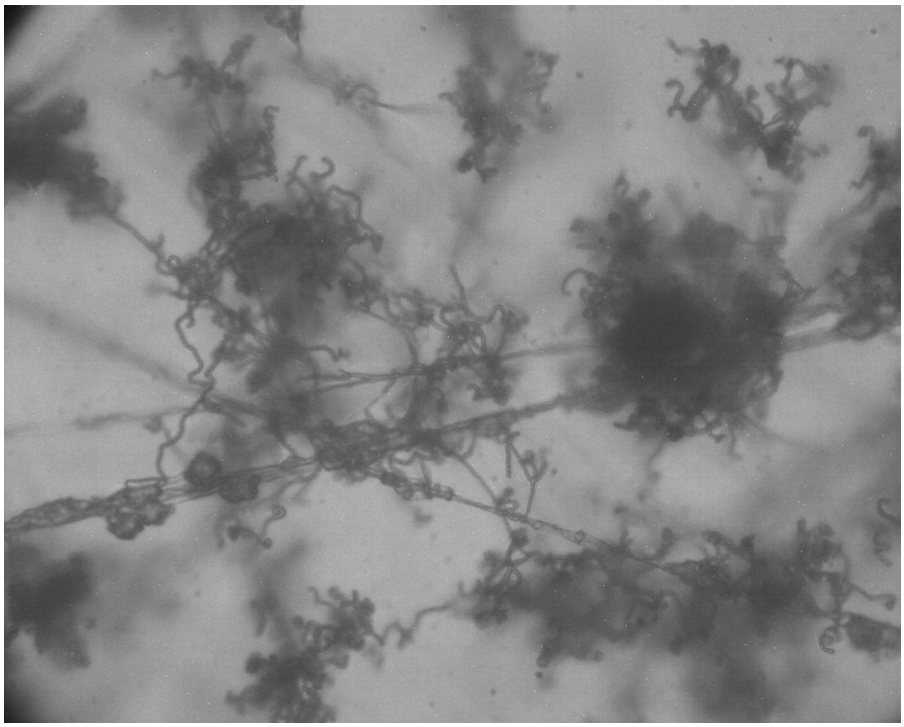

**Figure 3 Actinomycetes hiperparasite on pathogen Fusarium verticillioides(600x)**

Biochemical antagonism and hiperparasitism was evidenced by optic microscopy examination of interaction zone between *Trichoderma viride* and the pathogen.

*Trichoderma viride* developed coiled hyphae around pathogen and fed with its hyphal content by haustoria (Fig. 4).

Previous research (Cornea et al., 2009) evidenced a similar mechanism of antagonism for *Trichoderma* isolates from soil by producing haustoria that penetrated hyphae of pathogen *Botrytis cinerea.*

Double control mechanisms included hiperparasitism and biochemical antagonism when different *Trichoderma* isolates assayed against mycotoxigenic *Fusarium* spp. Both living strains and culture filtrates containing metabolites acted antagonistic against pathogens (Matei et al., 2014).

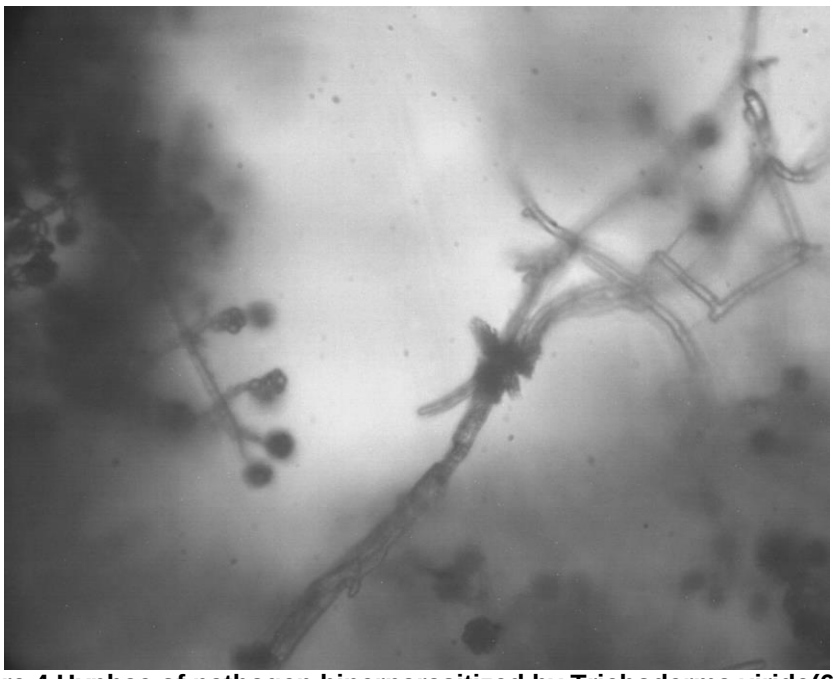

**Figure 4 Hyphae of pathogen hiperparasitized by Trichoderma viride(600x)**

Results from the present research are in concordance with other data from literature reporting that microbial character of soil suppressiveness was conferred by antagonists belonging to the actinomycetes, pseudomonads or to fungal strains of *Trichoderma* (Weller et al., 2002; Bonilla et al., 2012).

## CONCLUSIONS

Calcic chernozem from Enisala presented high densities of bacteria and fungi in surface horizon and moderate towards less aerated layers from the bottom of soil profile.

Global physiological activity of microflora followed the same pattern, with higher values af $CO_2$ released in surface than in subiacent horizons.

Bacterial communities were dominated by genus *Pseudomonas* with fluorescent and non-fluorescent species and by various actinomycetes.

Fungal communities were mainly represented by associations of ubiquitorious species from genera *Penicillium*, *Mortierella* and *Aspergillus*.

Cellulolytic species included fungal performant species from genera *Trichoderma*, *Mortierella*, *Stachybotrys*, *Chaetomium*, actinomycetes and bacteria *Cytophaga*.

The presence of an active antagonistic microflora and the absence of soil-borne plant pathogens are arguments supporting the suppressive character of soil.

Mechanisms of biochemical antagonism and hiperparasitism were evidenced against test-pathogen *Fusarium verticillioides* confirming the microbial origin of suppressiveness.

## AKNOWLEDGMENTS

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