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Soils from HNV agriculture systems as source of microorganisms with antifungal activity

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Abstract

Soils from rural zones with high natural value (HNV) agriculture systems are an important source of beneficial microbial species that can be useful for various biotechnological purposes, such as transfer of suppressiveness against plant pathogens from suppressive to disease-inducing soils by using inoculation with antagonistic selected strains.

The main goal of the paper was to present the results of the research carried out on strains isolated from soil microbial populations in HNV agriculture system (Mureș county, Romania) responsible for specific suppressiveness against soil-borne phytopathogens. The dual culture method was used for assessing the mechanisms involved in antagonism against a plant pathogenic strain from genus *Fusarium*.

The global microbial activity measured as soil respiration was intense. Total counts of bacteria and fungi estimated by dilution plate were also high.

The community of heterotrophic aerobic bacteria included 13 species. Associations of fluorescent pseudomonads and actinomycetes were dominant and presented antagonistic activity against *Fusarium*.

Twenty fungal species presented cellulolytic capability evidenced by growth on culture media with cellulose as sole source of carbon. Over cellulolytic capacity, the selected isolate of *Trichoderma viride* presented antagonistic activity against pathogenic *Fusarium* strain. Both biochemical mechanism and hyperparasitism were evidenced as involved in its antifungal activity.

Keywords: microorganisms, antagonistic activity, *Trichoderma viride*, phytopathogenic *Fusarium*, soil suppressiveness

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Introduction

Agriculture of high natural value (HNV- High Natural Value) represents a recent (last two decades) concept describing those agricultural systems with practices allowing preservation of high biodiversity in Europe. This concept recognizes that preservation of biodiversity depends on traditional agricultural practices but also on applying specific agricultural practices in large zones of rural European space.

The soils in these HNV systems are an important source of beneficial microbial species that can be useful for various biotechnological purposes, such as transfer of suppressiveness against plant pathogens from suppressive to disease-inducing soils by using inoculation with specific selected strains.

Fluorescent and non-fluorescent species of *Pseudomonas*, actinomycetes, endospore-forming bacteria, fungi from genera *Trichoderma*, *Gliocladium* are known as responsible for inhibition of plant pathogens by various mechanisms (1, 2).

The goal of the present paper was to present the results of the research carried out on bacteria and fungi isolated from soil microbial populations in high natural value agriculture system (Mureș county, Romania) responsible for specific suppressiveness against soil-borne phytopathogen *Fusarium* and to assess the antagonistic capacity of *Trichoderma* strain in order to be selected as performant bioinoculants.

Materials and Methods

Soil location: Soil samples (0-20cm) collected from high natural value (HNV) agriculture system, habitat 6210, Pilot area 1, Apold, Mureș county, belonging to European network of protected areas, Nature 2000(NGO property), 524 m altitude, 46^0 8'4" N latitude and 24^0 49' 16" E longitude, under grassland without anthropic intervention (pH-8.39, humus-4.02%, N_t-0.125%, N-NO_3-7 mg x kg^{-1}, P_{AL}-15 mg x kg^{-1}, K_{AL}-205 mg x kg^{-1}).

Microbiological analysis of soil sampled in September 2017, after hay harvesting from high natural value farming system was performed by plating soil decimal dilutions on specific solid culture media, Topping for heterotrophic aerobic bacteria, Czapek for fungi and Stapp for cellulolytic microorganisms.

After 7 days incubation at dark, microbial colonies developed were counted and their density was reported to gram of dry soil.

Taxonomic identification of bacteria was done according to Bergey's manual (3).

Fungi identified according to Domsch and Gams (4) and Watanabe (5) determinative manuals on the basis of colony morphology and structural characteristics observed by optic microscopy.

The global physiological activities of microflora were determined by substrate induced respiration method (SIR) and expressed as mg CO_2 x$100g^{-1}$ soil (6).

According to the Methodology of microbiological analysis utilized in Romanian soil monitoring system (6), values obtained were interpreted following the criteria:

Total bacteria counts
< 10 x 10^6 viable cells x g^{-1} dry soil - low number
10-20 x 10^6 viable cells x g^{-1} dry soil - moderate number
>20 x 10^6 viable cells x g^{-1} dry soil - high number

Total fungal counts
< 50 x 10^3 cfus x g^{-1} dry soil - low number
50-100 x 10^3 cfus x g^{-1} dry soil - moderate number
100 - 200 x 103 cfus x g^{-1} dry soil - high number
>200 x 10^3 cfus x g^{-1} dry soil - very high number

Soil respiration
< 30 mg CO_2 x $100g^{-1}$ soil - low
30-80 mg CO_2 x $100g^{-1}$ soil - moderate
>80 mg CO_2 x $100g^{-1}$ soil - high

Antagonistic activity of microbial strains against *Fusarium* pathogen

The microbial antagonistic capacity and mechanisms involved were assessed by dual culture method (7) against soil-borne plant pathogenic strain from genus *Fusarium*. Colonies were monitored for morphological characteristics, sporulation, production of metabolites and the presence of interaction zone.

Micrographs were done to reveal by optical microscopy the aspects of antagonism and its nature.

Results

Microbiological analysis

Quantitative assays (total counts and global microbial activities)

Total microbial activity measured as soil respiration was 82.590 mg CO_2 x $100g^{-1}$ soil.

The total counts estimated by dilution plate method were 38.815 x 10^6 viable cells x g^{-1} dry soil for heterotrophic aerobic bacteria and 179.968 x 10^3 colony forming units x g^{-1} dry soil for microscopic fungi.

Table 1. Taxonomic composition of bacterial and fungal microflora in soil from Apold

Soil profile	Bacterial species	Fungal species
	*Pseudomonas fluorescens,* *Bacillus megaterium* *Bacillus cereus* var. *mycoides* *Bacillus cereus* *Pseudomonas acidophila* *Bacillus* sp. *Pseudomonas* sp. *Pseudomonas pseudogleyi* *Bacillus circulans* *Serratia marcescens* *Bacillus subtilis* Actinomycetes Series Albus and Griseus	*Humicola grisea* *Penicillium glabrum* *Trichoderma viride* *Penicillium janthinellum* *Paecilomyces marquandii* *Cladosporium herbarum* *Absidia spinosa* *Aspergillus terreus* *Mucor vesiculosus* *Fusarium oxysporum* *Aspergillus niger* *Verticillium* sp. *Aureobasidium pullulans* *Cunninghamella elegans* *Cladosporium sphaerospermum* *Stachybotrys chartarum* *Penicillium* sp. *Penicillium aurantiogriseum* *Geotrichum candidum* *Aspergillus fumigatus*

Taxonomic composition of soil microbial communities

Bacterial community included 13 species, dominated by fluorescent pseudomonads and actinomycetes from series Albus with antagonistic activity against mycotoxigenic strains of *Fusarium*, accompanied by representatives of genera *Bacillus* and *Serratia* (**Table 1**).

A number of 20 fungal species were identified (**Table 1**), known for their large adaptability and metabolic ability to decompose various substrates.

Many strains presented the ability to grow on Stapp culture media with cellulose as sole source of carbon (e.g. from genera *Stachybotrys, Aspergillus, Cladosporium, Cunninghamella, Paecilomyces, Penicillium, Humicola*).

Antagonistic activity of microbial strain against Fusarium pathogen

Over cellulolytic capacity (**Fig. 2**), the isolate of *Trichoderma viride* presented antagonistic activity against *Fusarium* strain with both biochemical mechanism and hyperparasitism involved, as evidenced by macroscopic and optic microscopy examination of interaction zone (**Fig. 1** and **Fig. 3**).

Morphology and sporulation pattern of phytopathogen were modified (development and sporulation delays, hyphal twisting).

Hyperparasitism was evidenced when *Trichoderma* developed its own structures around the hyphae of pathogen, emitted haustoria inside it to feed and formed pustules that overgrew *Fusarium* colony.

Biochemical interaction was revealed by the existence of yellow metabolites more intensely released in the inhibition zone, well visible on the back side of the Petri dish.

Discussion

The results of the research carried out demonstrated the existence of microbial species diversity in soil from HNV agriculture system (Apold, Mureș county), including thirteen bacterial and twenty fungal taxa. The isolate of *Trichoderma viride* with high cellulolytic abilities presented antagonistic activity against phytopathogenic *Fusarium* strain.

Our results are in concordance with data from research carried out on *Trichoderma* strains showing antifungal activity against various plant pathogens including *Fusarium* (8, 9, 10).

Results from literature reported that biocide formulations with antimicrobial metabolic components extracted from *Trichoderma viride* alone or from combinations with other fungi were efficient in reducing disease incidence and activating defense response of tomato plants against pathogens (11).

As shown in images of optic microscopy captured in interaction zone between the strains of *Trichoderma* and *Fusarium* from dual culture, the metabolites of antagonist visible on the reverse of Petri plate suppressed the growth and fructification of pathogen.

Later, the colony of *Trichoderma viride* overgrew *Fusarium*, forming haustoria, pustules and feeding with the content of *Fusarium* hyphae by enzymatic lysis of the walls.

Figure 1. Interaction zone with *Fusarium* overgrown by *Trichoderma viride.*

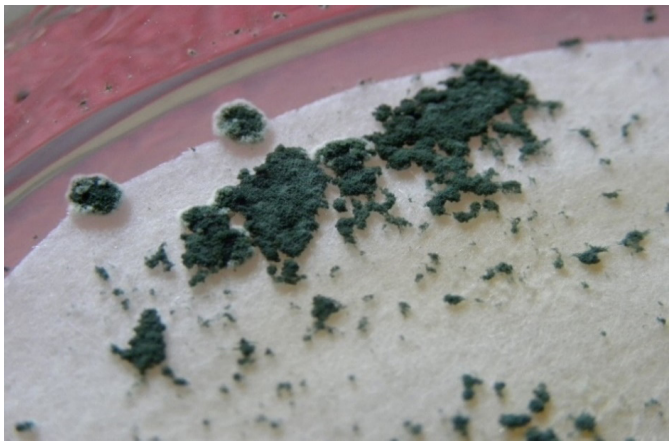

Figure 2. The cellulolytic capacity of *Trichoderma viride* evidenced by growth on Stapp medium with filter paper as sole source of carbon.

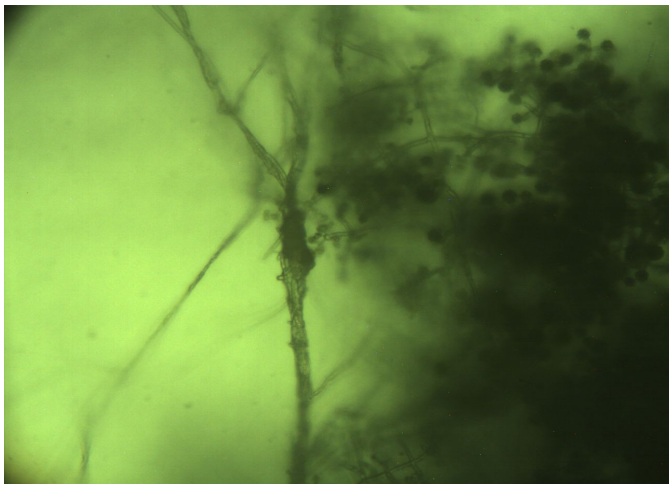

Figure 3. Hyphae and haustoria of *Trichoderma viride* hyperparasite on *Fusarium* hyphae.

Similar results with hyperparasitism and biochemical antagonism involved were reported for various *Trichoderma* strains that showed significant inhibitory effect against fungal pathogenic species (12, 13).

As in our previous research (14) with *Trichoderma viride*

strain SP456 that inhibited *Botrytis cinerea*, literature cites research with biopreparations of *Trichoderma* strains (e.g. *Trichoderma harzianum*-T22, well-known under commercial brand Root Shield) for the biocontrol of pathogenic fungi and explain their inhibitory properties by the ability to produce various enzymes and metabolites from the group of trichothecenes with antifungal properties (15).

Results of the present work evidenced the antifungal activity of *Trichoderma viride* isolated from soil in HNV agriculture system from Apold by biochemical antagonism and hyperparasitism and recommend it for utilization in biotechnological strategies of suppressiveness transfer against phytopathogenic fungi from genus *Fusarium*.

Conclusions

The well-developed and active microbial populations from soil in high natural value system under grassland, with 13 bacterial and 20 fungal taxa were dominated by fluorescent pseudomonads, actinomycetes and included many cellulolytic species of fungi.

Trichoderma viride presented cellulolytic and antagonistic activity against *Fusarium* strain with both biochemical mechanism and hyperparasitism involved.

Morphology and sporulation pattern were modified (delays, discoloration) and biochemical interaction was revealed by the existence of yellow metabolites more intensely released in the inhibition zone.

Hyperparasitism was evidenced when *Trichoderma* developed its own structures around the hyphae of pathogen *Fusarium*, emitted haustoria inside to feed and formed pustules.

Results of *in vitro* assays recommend the *Trichoderma viride* strain for biotechnological purposes, as antifungal agent in soil inoculation with microbial consortia for the transfer of suppressiveness against pathogen *Fusarium*.

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Conflict of interest statement

The authors declare that they have no conflict of interest.

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