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THE ROLE OF BIOSYNTHESIS HUMIC-LIKE PRECURSORS IN SOIL PROCESSES DYNAMICS

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Abstract

Microorganisms are involved in biosynthesis of the exogenous compounds with role in the dynamics of edaphic processes. A variety of such biosynthesized exometabolites, such as enzymes, phenols, carbohydrates, proteins, can be released into the soil, where they undergo biochemical interactions, form precursors involved in the synthesis of complex polymers and in determining a priming effect of biogeochemical processes. The research focused on the influence induced by humic precursors, extracted from the previously selected C1-C4 consortia, on the dynamics of bioprocesses in two soil types (Albic Luvisol and Fluvisols), respectively on enzymatic activities, biomass evolution, soil respiration and nitrifying microflora. Exometabolites from the C4 consortium showed the greatest diversity and complexity as humic-like precursors, followed by those the C3 consortium. In Albic Luvisol, the qualitative differences induced by precursors from consortia are well highlighted in the fulvic acid (FA) chromatograms. Enzymatic activity, DNA content, biomass and potential respiration level were influenced differently by the precursors in the C1-C4 consortia. Qualitative and quantitative analyzes for phenols and polysaccharides showed the influence of precursors on edaphic bioprocesses, in close correlation with soil type.

Key words*: chromatograms, exometabolites, humic-like precursors, microbial consortia, soil processes.*

INTRODUCTION

Several ways in which humic substances are formed are based on published theories based on lignin modification, amino acid-quinone interaction, microbial synthesis of aromatic compounds (Picart et al., 2017; Schutyser et al., 2018; Xu et al., 2018; Wang et al., 2020; Yang & Antonietti, 2021).

Each theory describes complicated biotic and abiotic reactions in which a variety of organic compounds, such as phenolic compounds, carbohydrates, and nitrogenous protein substances, are resynthesized to form large complex polymers (Zhang & Wang, 2020).

The variability of the molecular characteristics of humic substances is mainly due to humic precursors and the environmental conditions in which humic substances are formed (Madsen, 2011).

The biochemical processes in the cells of microorganisms active in the soil produce a mixture of metabolites that reflects the level and complexity of their metabolic activity. Exometabolites represent the set of metabolites released by microorganisms inoculated into the soil under the form of microbial consortia and which express a specific imprint, depending on their own composition (Romano et al., 2014; Fiore et al., 2015; Johnson et al., 2016). The diversity of exometabolites is much higher than theoretically anticipated, the results highlighting the possibility of biosynthesis of thousands of molecular masses (Matei et al., 2016a; Dickey et al., 2021). Thus, in addition to the anticipated exometabolites, new compounds appear in a large proportion in metabolic pathways, with molecular masses and unknown elemental composition.

These exometabolites are thought to form and may be released due to excess metabolic rate caused by the growth of microorganisms in the presence of abundant sources of carbon.

However, under normal conditions, in addition to signaling exometabolites (Hartmann & Schikora, 2012), vitamin and vitamin precursors (Sañudo-Wilhelmy et al., 2014; Johnson et al., 2016), siderophores (Linnik,

2020), 3-acetic acid indole plant hormones (IAA) (Wienhausen et al., 2017) and many other metabolites are released as humic-like precursors in the adjacent environment. Between soil microorganisms, the exchange of exometabolites and precursors is a common pathway due to extensive auxotrophy, with the growth of organisms themselves depending on the concomitant supply of exometabolites and mutualistic interactions between microorganisms (Li et al., 2019).

Also, the loss of some genes and the division of the ecological niche can be important factors in the co-evolution of auxotrophs but also the exchange of precursors can have profound implications.

Simplifying or adapting the genome of the selected microbiome correlates / includes the presence of several exometabolites and beneficial precursors for other microorganisms in the ecological niche, which promote the growth of auxotrophs and prototrophs, possibly because it does not involve providing energy for their synthesis (Mas et al., 2016; Estrela et al., 2016).

The idea of using microbial consortia is developing because such consortia through their exometabolites can fulfill / influence the performance of functions in the soil, more complicated than native populations, can be more resistant to environmental fluctuations and can intervene in the dynamics of soil processes (Tang et al., 2021).

In general, setting up consortia for the production of specific compounds remains a challenge.

We are looking for models and methods that allow the realization of consortia that produce various exogenous compounds involved in modifying edaphic processes and that influence the dynamics of humification, by integrating carbon from humic precursors, by determining a priming effect of biogeochemical processes, which can control C dynamics in soils.

The research aimed at the biostimulatory/ inhibitory influence induced by humic-like precursors, biosynthesized by the consortia of C1-C4 microorganisms previously selected, on the dynamics of bioprocesses in two soil types, respectively on enzymatic activities, humification, biomass and soil respiration, microflora, the content of phenols and polysaccharides.

MATERIALS AND METHODS

The Soils - Albic Luvisol and Fluvisols, type diagnosis in agreement with World Reference Base for Soil Resources (2014), had as agrochemical characteristics: pH 5.86, Humus (%) 1.74, Nt(%) 0.060, C/N 19.6, P_{AL} 35 ppm, K_{AL} 70ppm for Albic Luvisol and pH 8.36, Humus (%) 1.26, Nt(%) 0.106, C/N 8, P_{AL} 78.6 ppm, K_{AL} 126.6 ppm for Fluvisols.

Humic-like precursors biosynthesized by the C1-C4 consortia (Matei et al., 2016b), were extracted according to the IHSS methodology (Swift, 1996), treated with fluorochrome and their distribution was highlighted, by specific ascending chromatography. Thus, photographic images were obtained at 350 nm UV illumination, through which a qualitative analysis was performed on some aspects related to the density of the biochemical composition, molecular complexity and weight distribution.

In the experiment, the influence of the composition of humic-like precursors, extracted from the C1-C4 consortia, on the dynamics of soil processes, was analyzed after a period of 90 days from the application of 20 ml of humic precursors/pot.

The bioassay pots contained two types of agricultural soils (Albic Luvisol and Fluvisols). Each pot with 300g soil/pot was incubated at 27^0C, at a constant humidity of 60% of the soil field capacity and maintained for a period of 90 days, under the same controlled conditions.

At the end of this period, soil samples were collected for analysis. Five replicates were used for each experimental variant.

Phenol oxidases (PO) activity was determined by using 0.1 g of soil, MUB solution, pH 2.0 and 200 µl of a 0.1 M ABTS solution. After incubation at 30°C for 5 min, the mixture was centrifuged at 12000 rpm at 4°C for 2 min and the oxidation rate of ABTS to $ABTS^{+\cdot}$ released in the supernatant was measured at 420 nm. Enzyme assays were performed in three replicates for each soil sample, and statistically analyzed, according to the Student test.

FTIR analysis was performed to identify the structural and functional groups present in the fraction of fulvic acid extracted from Luvosol Albic, under the influence of humic precursors from the C4 consortium. The fulvic acid fraction was mixed well with 200 mg dry KBr

and pellets were obtained for further investigation in region 4000-500 cm^{-1}.

DNA extraction from soils was based on lysis with a high-salt extraction buffer (1.5 M NaCl), extended heating of the soil suspension in the presence of sodium dodecyl sulfate (SDS), hexadecyltrimethylammonium bromide, and proteinase K.

Soil microbial biomass (SMB-C) was determined under Romanian National Standard - SR-ER-ISO-14240-1-(2012)-Soil quality - Determination of soil microbial biomass.

Soil respiration was determined by the method of respiration induced by the addition of a substrate. The method measures the amount of CO_2 released by the microflora due to the use of the substrate and the results were expressed in mg CO_2 x100 g^{-1} dry soil (Ștefanic, 1991)

Nitrobacter count in the soil sample was estimated using MPN. The enriched soil is transferred to a liquid medium containing $NaNO_2$, incubated and shaken at 25°C. The culture was transferred to a fresh medium using a 1% inoculum, after eight passages, filtered through a membrane to trap the cells. There were washed, transferred on a fresh medium, again six passages on fresh medium, followed by filtration.

The tubes medium contain salts of potassium, magnesium, trace elements, iron and bromothymol blue as indicators.

The change in color of the tubes from blue-green to yellow indicates the oxidation, dilutions indicated by the presence of NO_2 and the population estimation, according to the tables.

The Phenolic content of the samples collected from the two soil types were analyzed by passing on liquid media, incubated at 35^0C for 48 hours, centrifuged at 10,000 rpm for 30 minutes.

The supernatant was filtered through microfilters and applied in quantities of 30 µl for wells from the agarized medium, used to highlight the phenolic compounds in the soils. Petri dishes with extracts were incubated, analyzed, photographed and semi-quantitatively evaluated for phenolic content by determining the diameter of the stained area.

The Polysaccharidic content was determined for evaluation of the biosynthetic capacities of the polysaccharide-type by soils microflora under the influence of precursors from C1-C4 consortia, on Petri dishes with a culture medium having an accessible carbon source. Petri dishes inoculated with extracts belonging to microbial consortia were incubated at 30°C for 24 hours.

Polysaccharide biosynthesis by capable soils microbiota, around the discs, was observed and photographed.

Quantitative determinations of polysaccharide content in the two soil types, under the influence of humic-like precursors, synthesized by microbial consortia were made by the procedure of Lowe (1993) with modifications.

The absorbance reading at 490 nm on the spectrophotometer was performed using distilled water instead of standard, as the control.

The evaluation of the hydrolyzed samples was performed according to the standard procedure, by performing the calibration curve and calculating the regression.

The results were expressed as total polysaccharide and statistically analyzed, according to the Student test.

RESULTS AND DISCUSSIONS

The entry of additional carbon from humic-like precursors, biosynthesized by the previously selected C1-C4 consortia, results in changes in soil organic matter dynamics and induces a priming / intensifying effect of biogeochemical processes by which C dynamics can be controlled in soils.

Significant taxonomic differences of the C1-C4 consortia, even when selected for effectively control of some processes in the soil, may induce performing different carbohydrate fermentation, have profoundly different biosynthetic capabilities, increasing the level of only certain activities (e.g. proteolytic), as well as producing harmful metabolites or amino acids.

The compositional and metabolic changes of the consortium microbiota are strongly associated with the predominant functions they perform, which may suggest links between normal / altered microbial metabolism and the level of functioning and health of the environment on which they will act biochemically as a product.

Taxonomic changes and quantitative increases in exometabolites may be associated with functional influences or disturbances of edaphic processes.
The result of the interspecific interactions of the microorganisms in the consortia and those dependent on the composition of the culture medium determines the spectrum of the consortium's own composition.
Systemic detection of the evolution of favorable / harmful microbial metabolites may also suggest the existence of mechanisms by which the biosis / dysbiosis of the consortium microbiota directly contributes to the cause of the impact on the dynamics of soil processes.
Thus, the exometabolites biosynthesized by the C4 consortium had the greatest diversity and complexity of humic-like precursors, close to that of the C3 consortium. In the case of consortia C2 and C1, chromatograms (a and b) show slow trends of complexation, possibly due to the inability to biosynthesize intermediate compounds or due to the self-generation of biochemical incompatibilities (Figure 1).

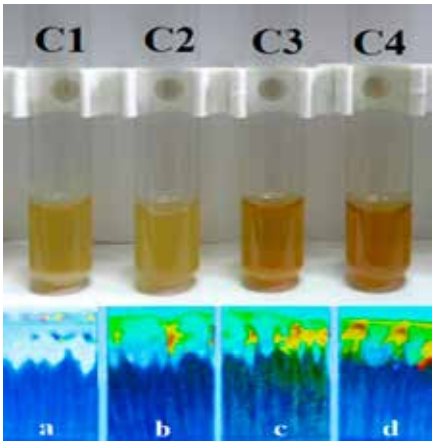

Figure 1. Humic-like precursors in aqeous solution, extracted from consortia C1-C4 and coresponded ascendant specific chromatograms
a) C1, b) C2, c) C3, d) C4

In Albic Luvisol, the fulvic acid (FA) content was 102 mg FA L^{-1} in the control soil, untreated with humic-like precursors and 134 mg FA L^{-1} in the same soil influenced by the application of precursors from C4 consortium, at the end of the experimentation period (90 days).

The qualitative differences between the precursors from the C1-C4 consortia, in terms of the influences induced by the FA content of the same soil type, were also significant in the case of the C3 consortium (128 mg FA L^{-1}). The influences induced by the FA content of Albic Luvisol by humic-like precursors from the C1 and C2 consortia were not significant compared to the control at the end of the experimental period.
The qualitative differences, induced by precursors from the C1-C4 consortia appear well highlighted in the specific ascending chromatograms of FA from Albic Luvisol, at the end of the 90 days.
They reveal accumulation areas with different densities, different concentrations and distributions of the compounds, specific influences of the precursors through which the direct contribution of each consortium is highlighted (Figure 2).

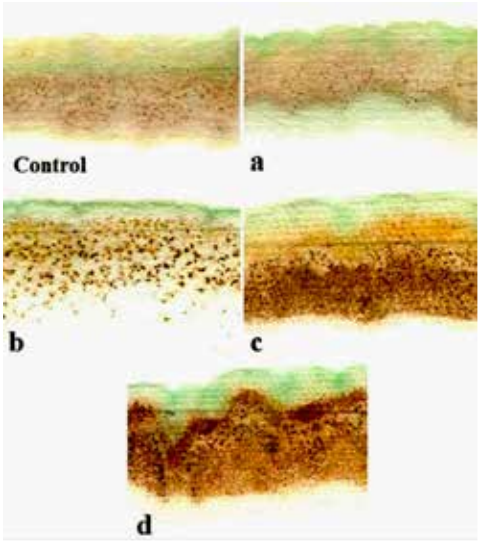

Figure 2. Chromatograms show qualitative differences in fulvic acid (FA) content due to the influence of humic-like precursors from a) C1, b) C2; c) C3; d) C4 and introduced into Albic Luvisol

In Fluvisols, FA content, determined under similar conditions, was 92 mg FA L^{-1} in control soil and 97 mg FA L^{-1} in humic-like precursors from the C4 consortium, determined at the end.
In terms of changes induced to the content of FA by precursors from the consortia, the

differences were not significant compared to the control.

However, there are qualitative differences, well highlighted in the chromatograms, in the variants in which the compositions of the alcoholic subfractions of FA are analyzed. Thus, these qualitative differences were highlighted between humic-like precursors from the consortia compared to the untreated control.

The chromatograms show the organic accumulations, after 90 days from the treatment, induced in the composition of the alcoholic subfraction of FA, by introducing the precursors from the C4 consortium (Figure 3).

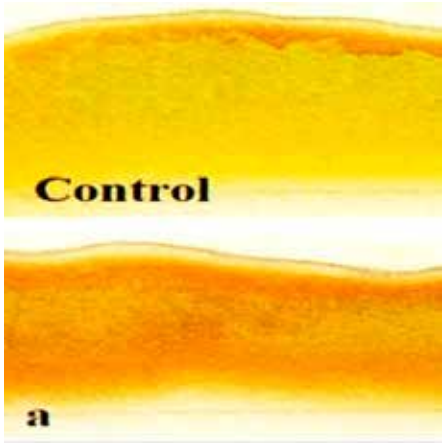

Figure 3. Qualitative differences between the alcoholic subfractions of FA in the humic-like precursors treated variant derived from C4 (a), compared to the untreated control in Fluvisols

The level of phenol oxidase activity determined in Albic Luvisol and in Fluvisols reflects the influence of humic-like precursors, coming from the C1-C4 consortia, on the evolution of the enzyme activity.

In Albic Luvisol, at the end of the experiment, the phenol oxidase activity in the control not treated with precursors was 0.19 $Ug^{-1}DM$ and in the variant treated with humic-like precursors from the C4 consortium it was 0.51 $Ug^{-1}DM$.

The level of enzymatic activity determined in the soil differs significantly between the variants treated with precursors from C2, C3 and C4 as well as in relation to the control variant. The enzymatic activity does not differ significantly from the control in the case of the influence exerted by the precursors from the C1 consortium.

In Fluvisols, the phenol oxidase activity in the control not treated with precursors was 0.16 $Ug^{-1}DM$ and in the variant treated with humic-like precursors from the C4 consortium it was 0.42 $Ug^{-1}DM$ at the end of the experiment.

In this soil, the level of enzymatic activity differs significantly between the variants treated with precursors from C2, C3 and C4 and in relation to the control, as in Albic Luvisol but its level of activity was lower.

The influence exerted by the precursors of the C1 consortium is not significant compared to the enzyme level reached in the control.

The activity of phenol oxidase (PO) in the two soils, under the influence of precursors biosynthesized by C1-C4 consortia, was determined because these enzymes also catalyze the oxidation of recalcitrant aromatic compounds such as lignin (Cullen & Kersten, 1996).

The PO activity also releases free radicals / quinones that can be involved in the synthesis of humic polymers, in the biodegradation processes but also in the evaluation of the dynamics of the edaphic processes, as sensitive indicators regarding their evolution. (Dec et al., 2003; Farnet et al., 2004).

Because the PO enzyme is particularly sensitive, it can also be used as a bioindicator of soil quality, health and the degree of adaptation of soil microbial communities to variations induced in the composition of soil organic matter (Taylor et al., 2002).

Generally, edaphic enzymes mediate the processes of synthesis / degradation of soil organic matter and their activity have been well studied (Błońska & Lasota, 2013; Dove et al., 2020; Piotrowska-Długosz et al., 2021; 2022). Phenol oxidases reach the soil by excretion or lysis, and through their activity mediate the functions of humification and mineralization of carbon, as well as the export of dissolved organic carbon. In soils, these enzymes are less stable, associate with organic particles or interact with mineral surfaces.

Their level of activity increases depending on the pH, the content of particles of organic matter and recalcitrant compounds, the presence of secondary metabolites.

Also, changes in nitrogen content in soils influence the expression of phenol oxidases, and in turn, they correlate with the content of organic matter in soils.
Thus, favorable changes in the organic matter content lead to an increase in oxidative activity because the changed content requires a correlation with the average potential activities of the enzyme.
In addition, the potential activity of phenol oxidase, according to the multiple regressions that takes into account the evolution of pH, temperature, precipitation, is considered to represent up to 37% of the variation of soil organic matter (SOM) content.
In addition, these factors interact and create positive / negative feedback on the dynamics of organic matter in the soil (Sinsabaugh et al., 2008). (Figure 4)

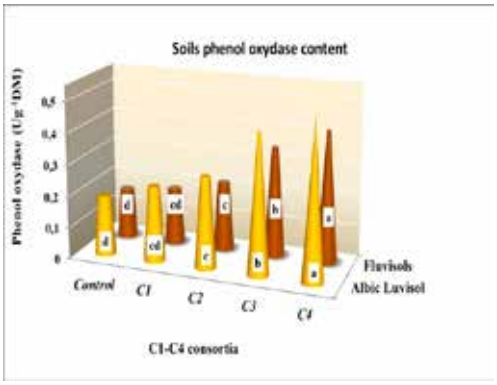

Figure 4. Soils phenol oxydase content under the influence of humic-like precursors from C1-C4 consortia

DNA extracted from Albic Luvisol and experimental variants treated with humic-like precursors from the four consortia was evaluated for purity and yield based on absorption ratios at 260/230 nm (DNA/humic acids) and 260/280 nm (DNA / protein).
The high absorption ratio of 1.234 at 260/230 nm indicated the purity of the DNA extracted from Albic Luvisol, in terms of humic acid contamination.
The value of the reports remained high (1.368 at C4) proportional to the level of microbial growth reached by each variant, at the end of the experimental period.
The ratio of 0.893, for absorption at 260/280 nm, indicates the purity in terms of protein contamination of DNA samples from Albic Luvisol.
The purity of the extracted DNA for protein contamination, extracted at the end of the experiment, with C4 precursors was 0.976.
To determine the nucleic acids in the soil sample, the DNA extract was exposed to ultraviolet light of 260 nm.
By spectrophotometric analysis, the DNA concentration in the soil was estimated at 13.2 µg g^{-1}, and under the application of microbial precursors, the DNA concentration was 14.06 µg g^{-1} at the application of C1 precursors and 16.75 µg g^{-1}, at the application of C4 precursors, at the end of the experiment.
The absorption ratio of 1.084 at 260/230nm indicated the level of purity of the DNA extracted from Fluvisols, in terms of humic acid contamination.
The value of the ratios was 1.102 for C1 precursors, 1.122-1.131 for C3, and C4, respectively.
To determine the concentration of nucleic acids present in the soil samples, the DNA from the Fluvisols was also quantified.
After exposure to 260 nm of the DNA extract from the soil, its concentration in the soil was estimated at 9.3 µg g^{-1}, and under humic-like precursors, the DNA concentration was 9.7 µg g^{-1} at the application of C1 precursors and 10.8 µg g^{-1} at the precursors from C4.
Under experimental conditions, at the end of the experiment, the influence of humic-like precursors on the dynamics of microbial reproduction, respectively on biomass accumulations was evaluated.
Thus, following the analyses, quantitative increases between 11-21% were found.
The biomass produced under the influence of the precursors of the selected consortium (C4), reached an average value of 262 mg C kg^{-1} soil, after 90 days from the treatment with humic-like precursors and represents the experimental variant with a 20.8% increase in the amount of microbial biomass relative to untreated Albic Luvisol biomass.
The biomass produced under the influence of the precursors of the selected consortium (C1) reached an average value of 226 mg C kg^{-1} soil, after 90 days from the treatment and represents the experimental variant with an increase of

only 3.6% of the amount of microbial biomass, in relative to untreated soil.

In the case of Fluvisols, the inoculation of the C4 consortium had a reduced influence through the biosynthesized precursors on the evolution of the microbial biomass content, an increase of only 11.25% (212 mg C kg^{-1} soil) compared to the control (190.5mg C kg^{-1} sol).

Also, the precursors of the C1-C3 consortia had a small influence on the evolution of the microbial biomass content, causing increases between 2.62-8.25%, compared to the control variant.

The physiological activity of the analyzed soil biota is reflected in the potential level of soil respiration.

Thus, in Albic Luvisol, the potential level of soil respiration determined 90 days after the introduction of the C4 consortium precursors was 128.85 mg CO_2x100 g^{-1} dry soil, compared to 94.32 mg CO_2x100 g^{-1} dry soil at the untreated control.

In Fluvisols, the potential respiration level determined under similar experimental conditions was 98.85 mg CO_2x100 g^{-1} dry soil, at the introduction of C4 consortium precursors, compared to 74.32 mg CO_2x100 g^{-1} dry soil, at the untreated control.

The FTIR analyzed the efficiency of the precursors from microbial consortia, in relation to the change in the qualitative and quantitative content of organic compounds in the composition of the fulvic fraction of Albic Luvisol.

Thus, the fraction of fulvic acids, under the influence of humic-like precursors of the C4 consortium, was analyzed by FTIR. In this case, the effect on carbon humification was monitored and changes in the functional groups in its composition were analyzed.

Generally, standard fulvic acids have peaks at 3497 cm^{-1} (OH bound to H), at 2925-2855 cm^{-1} (aliphatic CH stretch), at 2415 cm^{-1} (extended aliphatic CH stretch), at 1648 cm^{-1} (COO-, C=O of carbonyl and quinone), 1158 cm^{-1} (aliphatic CH).

In the case of FA from Albic Luvisol, the FTIR spectrum showed peaks at 3367 cm^{-1} (OH bound to H), at 2519 cm^{-1} (extended aliphatic CH range), at 1680 cm^{-1} (COO-, C=O of carbonyl and quinone), at 1578 cm^{-1} (NO, nitrogen compounds), at 1035 cm^{-1} (S=O, sulfoxides), at 966 cm^{-1} (OH deformation of COOH).

Thus, 90 days after the introduction of the precursors, the spectrum showed a strong absorption band at 3435 cm^{-1}.

Functional groups appear in addition to the untreated soil at 2309 cm^{-1} (O=C=O), 2218 cm^{-1} (C=C), 2047 cm^{-1} (N=C=S, carbodiimides), at 1941 cm^{-1} (C=C=C, alkene), at 1174 cm^{-1} (CO, ethers).

The weak band at 1725 cm^{-1} in the untreated and treated soil indicates a smaller number of COOH groups (Figure 5).

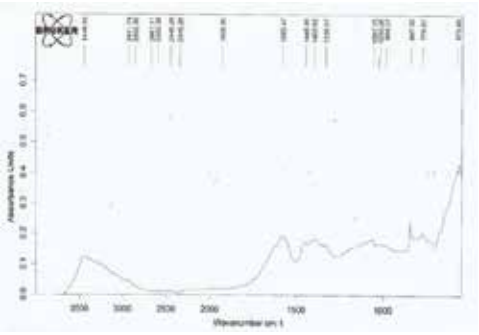

Figure 5. FTIR analysis of the influence of humic-like precursors from the C4 consortium on fulvic acids in Albic Luvisol

The influence of humic precursors on the activity and growth of chemoautotrophic microflora has been investigated *in vivo and in vitro*.

In vivo, Nitrobacter sp. multiplied after treatment with humic precursors from the C1-C4 consortia, reaching at the end of the period analyzed in Albic Luvisol at densities of 7.3 × 10^3 and 5.8 × 10^3 cells g^{-1} dry soil in Fluvisols under the influence of the precursors from the C4 consortium.

The lowest density of 2.6 × 10^3 cells g^{-1} dry soil was determined in Albic Luvisol, and 1.8 × 10^3 cells g^{-1} dry soil in Fluvisols, respectively, under the influence of the precursors from the consortium C1.

At the end of the analyzed period, the density values of the chemoautotrophic microflora were intermediate, in relation to the density values reached by the C4 and C1 consortia, in the two soil types, for the C2 and C3 consortia. Research on the evolution of the growth and development of this microorganism, considered

mandatory chemoautotroph, under the influence of precursors biosynthesized by C1-C4 consortia, was performed because it can be inhibited by the presence of organic matter, and the results allowed a better assessment of the direct influence of such organic compounds, defined as humic-like precursors, on it.

Also, as a nitrifying microorganism and biofertilizer, convert ammonia, reduce the forms of nitrogen present in the soil, into the most oxidized forms of the nitrogen, respectively nitrate.

Through its activity it ensures the functioning of the proceses, the control of nitrogen losses by leaching/denitrification of nitrates from the soil.

In vitro cultures with other edaphic microorganisms isolated from Albic Luvisol (*Bacillus* sp., *Pseudomonas* sp., *Trichoderma* sp.), treated with humic-like precursors from the C1-C4 consortia, showed for each, significant improvement in growth, compared to untreated control.

Biosynthesized humic-like precursors have influenced the growth of autotrophic nitrifiers, most of the precursors used have stimulated bacterial and fungal populations in natural soils (Albic Luvisol, Fluvisols).

It was also observed that there were no microbial growths dependent on the humic-like concentration of the applied precursors, which may suggest that they are not mainly used as a source of carbon and nutrients by the microorganism.

The results complete the absence of information on the response of autotrophic nitrifying bacteria to the presence of prehumic compounds in the soil, under axenic conditions, with representative strains.

In fact, biosynthesized humic-like precursors possibly improve the soil, allowing nitrifiers to grow in a better aerobic environment, compared to humus obtained from leonardite or other sources, which causes the microaggregates in the soil to collapse (Kaya et al., 2020; Akimbekov et al., 2021).

The differences induced in the soil by biosynthesized organic compounds compared to those extracted from different organic materials can be attributed to the divergent effects induced by their composition on some physico-chemical characteristics of the soil.

It can be considered that the influence of humic-like precursors in the soil depends more on their superficial characteristics, through which they can modify the permeability of cell membranes, causing a better absorption of both mineral nutrients and oxidizing substrates that produce energy. Microbial consortia, by excreting humic-like precursors, could control the decomposition rate of organic matter in the respective soil, or can intervene in the stabilization of organic carbon and in the dynamics of the circuit of the elements.

Phenols, as secondary metabolites that decompose slowly in soils compared to organic matter, show useful recalcitrance in protecting carbon stocks. Also, the microorganisms from soils can influence the form in which phenols can exist in the soil, starting from the reduction of dissolved forms (free in soil solution and exposed to degradation), to stimulating the involvement of phenols in the adsorbed forms in soil/proteins, or in polymerized forms (present in the composition of humic substances).

The phenolic content of the two soils, depending on the influence of humic-like precursors from C1-C4 consortia was directly proportional to the halo diameter and the intensity of the colored area, around the well. Thus, the highest phenols content in Albic Luvisol was synthesized under the influence of precursors from C4 consortium (ø28.5 mm), followed by those from C3 consortium (ø 17.5mm), by the C2 consortium (ø14.5mm), and the lowest phenols content obtained in this type of soil was obtained from the influence of humic-like precursors from C1 consortium (ø8.5mm). The phenolic content of Albic Luvisol synthesized under the different influence of the precursors from C1-C4 consortia and the halo of diffusion of the phenolic content can be observed from the soil samples analysis (Figure 6).

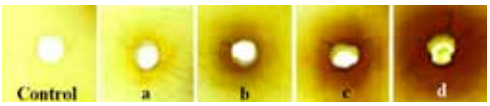

Figure 6 The content of phenolics of Albic Luvisol, under the influence of humic-like precursors from control and consortia a) C1, b) C2, c) C3, d) C4

The content of phenols in Fluvisols synthesized under the influence of humic-like precursors of the C1-C4 consortia followed the evolution of the content from the Albic Luvisol, but smaller diameters were obtained for the diffusion halos, in the analyzed soil samples, possible due to the reduced quantities of synthesized phenolic content. To evaluate the influence of precursors from C1-C4 consortia on the polysaccharide content of the two soils, soil extracts were inoculated on sterile filter discs arranged in Petri dishes to a semi-quantitatively evaluation of specific evolution of edaphic microflora capable of such biosynthesis in Albic Luvisol. The chemical and biological synthesis processes in soils were strongly influenced by humic-like precursors from the C1-C4 consortia, possibly due to better adaptation or higher compatibility with the biochemical composition or with endemic microbial structure of the soil.

The precursors from C4, present in Albic Luvisol, stimulate endemic microbiom to biosynthesize a higher polysaccharides quantity (Figure 7).

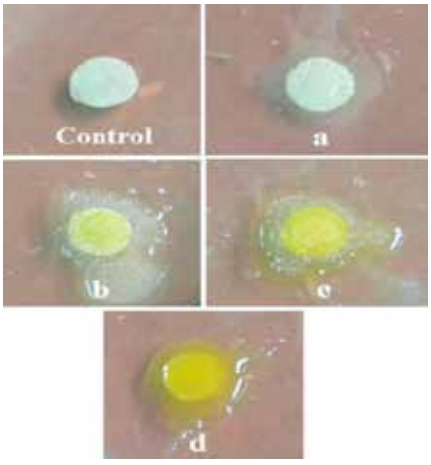

Figure 7. Semi-quantitative evaluation of the specific evolution of the edaphic microflora capable of polysaccharide biosynthesis, in Albic Luvisol, under the influence of humic-like precursors from consortia a) C1, b) C2, c) C3, d) C4

In Albic Luvisol were higher amounts of polysaccharides than those of Fluvisols, but the quantities differ depending on the type of consortium.

The polysaccharide content, in the two soil types, under the influence of humic-like precursors, from C1-C4 consortia was analyzed.

Quantitative analyses allowed to be determined the highest content of polysaccharide compounds in Albic Luvisol, under the influence of humic-like precursors from the C4 consortium (63.7 mg g^{-1} polysaccharides).

The differences are significant compared to the polysaccharides level in the control, and were significant differences between C4 and C1 consortia.

The quantitative differences, induced by the C2 and C3 consortia are not significant, in terms of the evolution of the polysaccharide content. In the Fluvisols under the influence of humic-like precursors from C4 consortium, the determined polysaccharide content was 48.5 mg g^{-1} polysaccharides.

The differences are significant compared to the level of control for the C2, C3 and C4 consortia.

The polysaccharide content determined in Fluvisols was the least influenced by the C1 consortium (36.5 mg g^{-1} polysaccharides), the differences being not significant in compare with control (Figure 8).

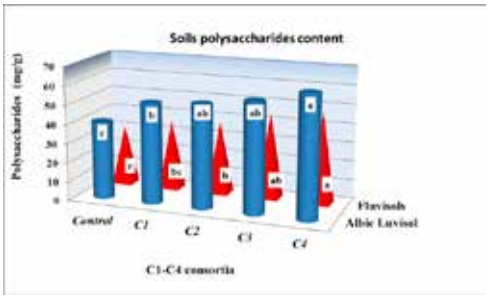

Figure 8. The polysaccharides content in two soil types, under the influence of humic-like precursors, from consortia C1-C4

CONCLUSIONS

Exometabolites biosynthesized by the C4 consortium show the greatest diversity and complexity of humic-like precursors, followed by the C3 consortium, and C1 and C2 show low diversity due to reduced biosynthetic capacity or incompatibilities.

In Albic Luvisol, the fulvic acid (FA) content was significantly influenced by the application of humic-like precursors from the C3 and C4 consortia.

The qualitative differences induced by humic-like precursors from the C1-C4 consortia are well highlighted in the FA chromatograms of Albic Luvisol by accumulation areas with different densities, concentrations and different distributions of specific compounds, highlighting the direct contribution of each consortium.

Qualitative differences are highlighted in the chromatograms, in the comparative analysis with the control for the composition of the alcoholic subfractions of FA, more obvious in the case of the C4 consortium.

The phenol oxidase activity determined in Albic Luvisol and in Fluvisols reflected the level of influence of humic-like precursors, from the C1-C4 consortia, on the intensity and evolution of the enzyme activity.

The DNA concentrations in Albic Luvisol and Fluvisols were 13.2 $\mu g\ g^{-1}$ and 9.3 $\mu g\ g^{-1}$, respectively, and by applying precursors, the concentrations increased in all variants, and in the case of C4 precursors up to 16.75 $\mu g\ g^{-1}$ and 10.8 $\mu g\ g^{-1}$, respectively.

The biomass produced under the influence of humic-like precursors of the selected consortium (C4), reached an average value of 262mg C kg^{-1} soil, representing the variant with increases of 21% and respectively 11%, compared to the biomass from Albic Luvisol and respectively Fluvisols, both untreated.

The potential level of respiration, determined 90 days after the introduction of the C4 consortium precursors in Albic Luvisol, was 128.85 mg CO_2 x 100 g^{-1} dry soil, compared to 98.85 mg CO_2 x 100 g^{-1} dry soil in Fluvisols, untreated.

The phenolic semi-quantitative content from two soils, under the influence of humic-like precursors from C1-C4 consortia was the highest under precursors from C4, and the lowest from C1 consortium.

In Albic Luvisol, the polysaccharide content under the influence of humic precursors differs significantly between the consortium variants, between them and the control and in Fluvisols the differences are not significant compared to the control, for the C1 consortium.

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