ENHANCING SOIL SUPPRESSIVENESS WITH CONJUGANTS REALIZED BETWEEN MICROBIAL SIDEROPHORES AND A FULVIC ACID FRACTION

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Abstract

The paper aims to select probiotic microorganisms capable of producing siderophores for iron chelation and to make conjugates with the water-soluble fulvic fraction of Mollic Gleysol (WRB), (0–20 cm). The biosynthetic performances of 5 strains of probiotic bacteria from the collection, belonging to genera Lactobacillus (LAB 41, LAB 62, LAB 57, LAB 83, LAB 69), isolated from different soil types, were evaluated in terms of siderophores production (CAS method), type (Arnow and Csaky tests) and complexation capacity. The probiotic strains produced amounts of siderophores between 97–158 μmol L^{-1} in 48–96 hours, of catecholate and hydroxymate type and of both types in the case of LAB 62, LAB 69 and LAB 83 strains. LAB 83 strain had the highest iron chelating capacity. The inhibition capacity of the conjugates was tested and evaluated on 3 phytopathogenic fungal isolates (P. expansum, A. flavus and A. ochraceus). Probiotic strains are promising for the purpose of producing siderophores but also for antifungal effect when these act as conjugants with a fulvic acid subfraction.

Key words: siderophores, probiotic bacteria, fulvic acid subfraction, antifungal effect, conjugants.

INTRODUCTION

In the soil and rhizosphere of plants, the balance between saprophytic and phytopathogenic microorganisms is the determining factor in defining the state of health. In some soils, imbalances favoring the saprophytic component occur so that diseases caused by *Fusarium* spp., *Alternaria* spp, *Rhizoctonia* spp, become unimportant or phytopathogens fail to adapt to those conditions. Such disease-suppressing soils are still insufficiently defined regarding the specific microbial components antagonistic to phytopathogens, as well as the environmental conditions favoring their proliferation. New and relatively effective ways of using agents or their metabolites, in achieving biological control, in order to promote soil and root health, by harmonizing the numerous factors involved in the generation of rhizosphere dynamics, have been constantly developed.

Also, due to the complexity of the interactions of the interdependent factors, simultaneous techniques of stimulation/stabilization of antagonist populations, of root-microbe-microbe interactions as well as intentional alteration of the rhizosphere were used to create unsuitable conditions for phytopathogens.

Suppressive soils offer a natural defense to plants, through the soil microbiota, which constitutes a first line of defense against phytopathogens (Schlatter et al., 2017). In such soils, due to the composition and activity of the microbiota, biosynthesized metabolites, phytopathogens do not establish, cause or not disease, or initially establish in the soil and cause the disease, but then it decreases in intensity, even if the phytopathogens persist in the soil (Weller et al. 2002).

Their combinations generate "general" or "specific" suppressions that ensure in the soil the increase in the level of competitive and collective antagonistic activities of the total soil microbiome, generating competition with phytopathogenic agents. The induced suppressiveness aims to stimulate the pre-existing characteristics in soils, specific to each type of soil and non-transferable.

Probiotic bacterial strains or their siderophores, inoculated in soils with phytopathogens, could intervene by increasing its suppressiveness. Thus, the suppression of various diseases caused by fungal phytopathogens belonging to

the genera *Fusarium, Aspergillus, Alternaria, Verticillium* can be obtained by the intake of siderophores from probiotic microbial agents or from their conjugants, through interventions on the efficient complexation of iron (III) in the soil, by causing its unavailability for phytopathogens and inhibiting their growth.

The impact of siderophores on soil iron biogeochemistry may be influenced by the presence of other soil iron-binding compounds such as humic substances (Tipping et al., 1993; 2011; Milne et al., 2011; Niehus et al., 2017; Slagter et al., 2017; Whitby et al., 2020). Studies in the field have highlighted the fact that the distribution of binding sites in organic matter is heterogeneous, relatively similar to those observed in humic substances (Zhu et al., 2021).

Siderophores can act synergistically with fulvic acids (FA) which also function as ligands for the release of Fe from Fe(III) oxides. By combining the two compounds, in different proportions, conjugates can be formed that can adsorb metals more efficiently and release them in larger quantities. The synergistic relationships between siderophores and the water-soluble subfraction of fulvic acids (FA) or the effect of their conjugates, highlight new possibilities regarding the efficiency of Fe (III) acquisition mechanisms in order to ensure its availability for plants and microorganisms, but also to enhance the antimicrobial effects, through which can intervene in the achievement of an effective biocontrol of edaphic phytopathogens.

In this study, the qualities of five probiotic strains of *Lactobacillus* spp. were evaluated regarding the production of siderophores, the formation of conjugates with the water-soluble subfraction of fulvic acids extracted from Mollic Gleysol, and their antifungal character against three fungal species was analyzed (*P. expansum, A. flavus, A.ochraceus*), for a possible application in soils in order to increase their suppressive capacities.

MATERIALS AND METHODS

Screening and growth of probiotic strains. The probiotic bacterial strains come from the laboratory collection and were isolated by the method of serial dilutions using MRS (De Man Rogosa Sharpe) agar medium, from two types of soil according to WRB (Haplic Chernozem, Rendzic Leptosol). Plates were incubated at 27°C for 72 hours, identified morphologically and characterized by biochemical tests. The strains were preserved and maintained on MRS broth at 4°C. Morphologically, the strains LAB 41, LAB 57 presented rough, yellow, irregular surfaces and LAB 62, LAB 69 and LAB 83 presented smooth, white, circular surfaces, Gram-positive, by the Gram staining method.

Qualitative detection of siderophores production was performed using the CAS (Chrome Azurol S) assay according to Schwyn & Neilands (1987). The culture supernatant obtained was mixed in equal volumes with CAS reagent and the color change observed. The reference was prepared on the basis of the uninoculated medium, as a control. Through the qualitative determinations, the production of siderophores was estimated for a number of 12 isolates and of these, five probiotic bacterial strains, LAB 41, LAB 62, LAB 57, LAB 83, LAB 69, were selected for the following tests, due to their increased synthesis capacities.

Quantitative detection of siderophores. The chromium azurol S (CAS) method was used to estimate bacterial siderophore production due to its detection capability (Alexander & Zuberer, 1991). The culture media were centrifuged, the supernatant filtered through a 0.45 μm filter, stored at low temperature. Afterwards, 1.0 mL of filtrate was mixed with deionized water and 1 mL of test solution. For the reference solution, 1.0 mL of CAS solution was mixed with 1.0 mL of deionized water and for the zero absorption solution, 1.0 mL of CAS solution with 1.0 mL of deferoxamine, kept at room temperature, in the dark, 24 hours and read the absorbance at 630 nm. Siderophore production, according to the calibration curve, was expressed as $\mu mol \ L^{-1}$ deferral equivalent. For each probiotic isolate, samples were read in triplicate.

Determination of the type of siderophores. synthesized by the selected strains was carried out using the Arnow test, for the catecholate siderophore type and the Csaky test, for the hydroximate siderophore type.

Conditions for the synthesis of siderophores. Factors were similar for siderophore production in all strains (incubation time, pH, rpm,

inoculum volume). The incubation period was 96 hours using the CAS assay and pH for growth of strains in medium was 7-8 using identical molarities for HCl and NaOH. Agitation at 180 rpm and 2% inoculum volume was used for siderophore production.

Siderophore extraction. After completion of incubation period, cell mass from each five culture strains were separated by centrifugation at 10.000 rpm, 4°C for 15 min and supernatant was concentrated. Siderophores were extracted in two phases with phenol: chloroform, diluted with diethyl-ether and transferred to aqueous phase.

Complexation capacity assays. Siderophore complexation capacity was assessed following a method adapted from Villen et al. (2007). To a constant volume of culture filtrate, FeCl3 was added, the pH adjusted to 9.0, for 30 min. The obtained solution was centrifuged, filtered through a 0.45 µm pore membrane and the amount of Fe in the solution was determined and presented graphically.

The Fulvic water-soluble subfraction Fulvic fraction from Mollic Gleysol was extracted and fractionated by adsorption on activated charcoal and serial elutions with acetone, NaOH of the absorbed fulvic substances and with distilled water for separating water-soluble subfraction (Votolin et al., 2022).

Specific ascending chromatograms Quantitative/qualitative changes of fulvic subfraction and the degree of complexity reached was obtained by using specific ascending chromatogram adapted method (Mukadam et al., 2021).

The formation of image pattern of uniformity, shape, size and color could directly highlight the variation of composition of labile fulvic compounds, in a short period of time.

Conjugates siderophores-fulvic subfraction Siderophore-fulvic subfraction conjugates were designed to intensively depriving the phytopathogens from iron, as antimicrobial strategy, based on conjugants features, which were enabled for a more active iron uptake than individual components and for using as supplementary weapon in antifungal treatment.

Fluorescence of conjugates The siderophores-fulvic subfraction conjugants were treated with fluorochromes and their distribution was revealed (Wang et al., 2021). Thus, photographic images were obtained under UV illumination of 350 nm, which revealed the quantitative differences between conjugants, related to the density of the newly biochemical compounds, in close agreement with fluorescence-based affinity.

Antifungal activity. Agar well diffusion assay was used to detect the antifungal activity of the conjugates against phytopathogens (*P. expansum, A. ochraceus, A. flavus*) grown on PDA (potato dextrose agar) medium. The agar plate surface was inoculated by spreading a volume (1 ml) broth culture of the phytopathogen inoculum (10^3 ufc x ml^{-1}) over the entire agar surface, incubated at 27°C for 24 hours. 50 µl of conjugants were placed in agar wells, incubated at 27°C, five days and measured the diameter of inhibition zone around the wells.

RESULTS AND DISCUSSIONS

The colonies of bacteria developing a yellow halo on CAS medium (Figure 1) were selected and isolated from two types of soils (Haplic Chernozem, Rendzic Leptosol) for producing siderophore.
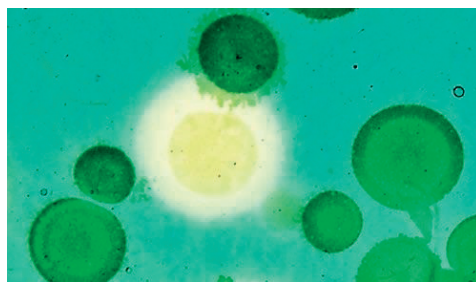

Figure 1. Isolation and selection of siderophores-producing bacteria (colony with yellow halo)

Initially, twelve probiotic bacterial isolates from the two soils were used for screening to siderophore production capacity, both qualitatively and quantitatively.

Five efficient bacterial strains were selected and accepted to be analyzed in terms of siderophore type, production and complexation capacity, in order to be used in increasing the suppressiveness of soils.

All efficient probiotic strains were grown at 27°C with shaking in MRS broth. Pre-cultures

were prepared in media with normal iron content.

The cell mass of the probiotic bacteria was separated, washed and re-inoculated in the same type of medium, but without optimal iron content. All operations were carried out during the exponential growth phase, to limit the transfer of iron from the iron medium, as it could intervene by inhibiting the production of siderophores. At the end of growth, iron was below the detection limit in the culture medium. All probiotic strains grew in the medium and had different doubling times. In the stationary phase of growth, LAB 41 and LAB 57 strains grew more slowly in the culture medium. Under the culture conditions, they showed a doubling time of 18 hours and were in stationary phase after 48 hours. The probiotic strains LAB 62, LAB 67 and LAB 83 grew faster in the culture medium and were in stationary phase after 24-36 hours (Figure 2).
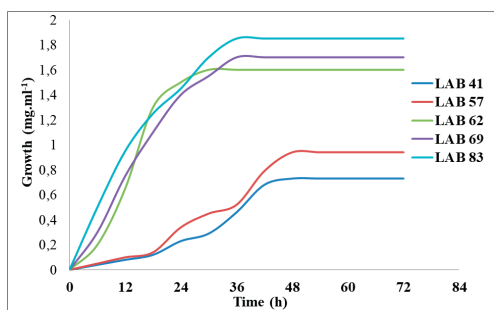

Figure 2. The growth of probiotic bacteria

In the first stage, qualitative screening of probiotic strains has been performed using CAS agar plates. So, all five strains that formed a yellow to orange zone around the well as seen in Figure 3 indicated a positive siderophore production. All strains tested were positive for siderophore production, the CAS agar method providing only an approximation and not an accurate quantification of siderophore production. In the agar well diffusion test, when supernatant of each probiotic broth culture were applied to the wells, the appearance of a halo yellow to orange around the wells indicates the production of siderophores.

The halo diameter varied from strain to strain and was considered direct proportional with the content of synthetized siderophores. The best performance were obtained by five probiotic strains which used forwards in making conjugates. The strain LAB 83 determined the highest diameter of the halo to 28.6±0.26 mm and the strain LAB 57 determined the lowist diameter of the halo to 12.4±0.11 mm. The probiotic strains LAB 41, LAB 62 and LAB 69 determined intermediate values of the halos diameters (Figure 3).
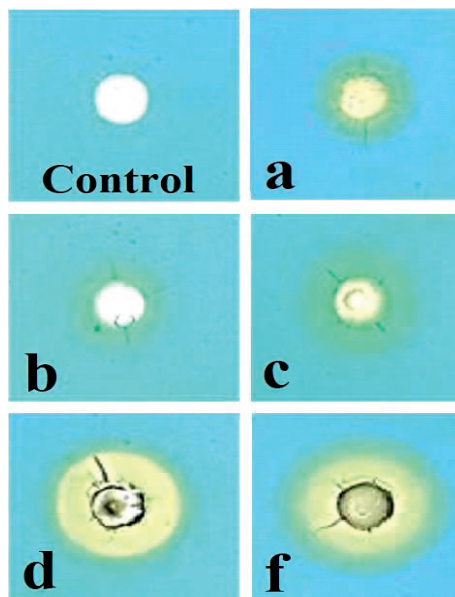

Figure 3. The halo diameter in direct relation with the content of synthesized siderophores by a) LAB 41, b) LAB 57, c) LAB 69, d) LAB 62, f) LAB 83

The determinations revealed that the LAB 83 strain was the most effective in terms of qualitative evaluation of siderophore production, compared to the other probiotic strains. Based on the diameters of the halo, the strains were grouped into three levels of siderophore production (Table 1).

Table 1. Siderophore production by probiotic bacterial strains and their estimation using qualitative analysis

Probiotic bacterial strains	Qualitative siderophores analysis
Control	-
LAB 41	++
LAB 57	+
LAB 62	++
LAB 69	++
LAB 83	+++

The production of siderophores is a specific feature of many groups of edaphic microorganisms, soil bacteria and fungi, but also phytopathogenic agents. The production of siderophores by the analyzed edaphic bacteria highlights an important feature of them that may suggest the possibility of involvement both in growth promotion and in the biocontrol of phytopathogens (Johnstone et al., 2015; Koh et al., 2015; Duar et al., 2017; Senthilkumar et al., 2021). Our results highlight that siderophore production occurs on a large scale by soil microbiota and many of the probiotic bacteria produce and utilize siderophores. Siderophore production of probiotic bacteria was monitored and compared, at different time intervals, using the CAS method. In the culture conditions tested, the concentration of siderophores determined in the culture filtrates of *Lactobacillus* spp. reached the maximum level at 96 hours, when they were in stationary phase. After this time interval, the level of siderophores remained relatively stable. According to the same model, siderophores synthesized in culture filtrates LAB 62, LAB 69, LAB 83 reached the maximum level at 48 hours. The concentration of siderophores in the filtrates was relatively stable after 32 to 48 h of growth, followed only by a variation in the values after 48 h. In the case of the LAB 83 strain, a further increase in siderophore production was observed even after 56 hours (Figure 4).
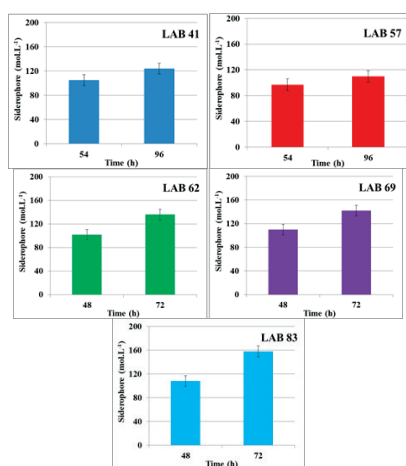
In the present investigation, the five probiotic bacterial isolates of *Lactobacillus* were tested for 2 different types of siderophore. All isolates (LAB 41, LAB 57, LAB 62, LAB 69, LAB 83) were positive in Arnow's test that detects the catechol type of siderophores. Three isolates (LAB 62, LAB 69, LAB 83) tested positive in Csáky's test which detects the hydroxamate type of siderophores (Table 2). No other determinations were made for other types of siderophores (carboxylate).

Table 2. Analysis of the siderophore-type produced by the probiotic bacteria

Probiotic bacteria	Arnow[a]	Csaky[b]
LAB 41	+	-
LAB 57	+	-
LAB 62	+	+
LAB 67	++	+
LAB 83	++	+

Catechol[a] and hydroxamate[b] - type siderophores were identified using Arnow[a] and Csaky[b]s tests, with ++ strong positive; + positive; − negative result

Hydroxamate siderophore units present a broad spectrum of antibiotic activity, through bactericidal units that can inhibit tRNA synthetase, similar to fungal ferrochromes, due to the presence of ferrochrome receptors in both Gram-positive and Gram-negative bacteria. These naturally occurring "Trojan horses" formed the basis for the development of synthetic antimicrobial conjugates (Ji et al., 2012; de Carvalho et al., 2014). Siderophores exhibiting hydroxamic acid groups for metal binding and containing additional cyclic structures have the potential advantage of conjugate stability over a wide pH range, enhancement of specific activities and high stability at low conjugate concentrations. Thus, trihydroxamate siderophores (deferoxamine B) act synergistically with fulvic acid, as a low molecular weight organic ligand, to release Fe from Fe (III) oxides. Desorption and release from conjugates can be influenced by the type of siderophore, the composition of the fulvic acids subfraction, as well as their combined effect.

Selected probiotic bacterial filtrates could be used to make siderophore conjugates for soil amendment. Thus, in order to assess the iron chelation potential of the culture filtrates of the selected bacterial probiotics and the influences

Figure 4. Siderophore production of probiotic bacteria using the CAS method

due to the culture conditions, they were subjected to complexation capacity analyses (Payne et al., 1993; Perry et al., 2015; Roosenberg et al., 2000; Souza et al., 2017). The results of these analyzes on the complexing capacities are presented in Figure 5. For strains LAB 62, LAB 69 and LAB 83, the initial slopes of the experimental data are close to 1 up to a [Fe] added/Siderophore ratio of about 1.1-1.2, indicating effective and good complexation to the amount of added iron. In the probiotic bacterial strains LAB 41 and LAB 57, delays are observed, the change from the slope = 1 appears in the [Fe] added/Siderophore ratio values of 2.4-2.7, showing an efficiency of approximately 33-45%. Deviations may be due to inefficient complexation in the pH range used, complexation of siderophores/chelating agents with weaker characteristics, to which the added iron cannot be efficiently complexed or may precipitate. The average of iron complexed compared to that of added iron, depending on the total concentration of siderophores, was determined by the CAS method. For each filtrate, based on the CAS values, the amount of effectively complexed Fe was estimated. For example, in literature was describes that *R. radiobacter* produces agrobactin, which complexes iron in a 1:1 ratio, in a hexadentate conformation; thus, a slope close to 1 would be expected as a result of high complex stability and consequent efficiency of complexation.

The observed results may be due to the different efficiency of iron chelation under the established conditions, as well as the presence of siderophores with different stoichiometry and stability. Thus, in the case of the filtrate of LAB 57, taking into account a CAS reading, we detect 97 µmol L^{-1} of dissolved iron upon the addition of about 262 µmol L^{-1} of Fe. A filtrate of LAB 83 was able to completely dissolve 158 µmol L^{-1} of Fe by adding 191 µmol L^{-1} of Fe. The observed results may be due to different iron chelation efficiency under the established conditions, stoichiometry and specific stability.

Fulvic acids and its water-soluble subfractions constitute novel antimicrobial molecules reported to have antifungal properties and the ability to bind and eliminate toxins in soil, such as heavy metals, making it a powerful detox ally compound.
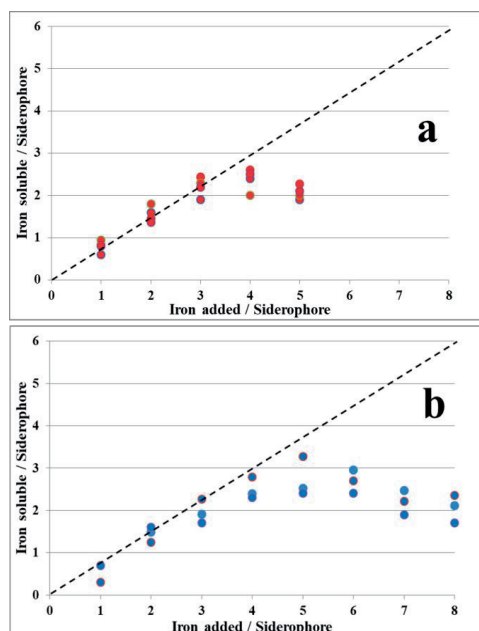

Figure 5. Estimation of the amount of effectively complexed Fe in relation to the values of Fe added / Siderophores, based on the CAS values a) LAB 83, b) LAB 57

The change in the structure of organic compounds in soils, especially of the fulvic acid (FA), determines intense interactions with the plant growth process and the activity of microorganisms.

There are relatively few studies on the antifungal activity of fulvic compounds, especially FA subfractions against plant phytopathogenic fungi.

Organic compounds (humic or fulvic precursors) extracted from soils, as well as synthetic ones show antimicrobial activity, compared to those extracted from peat or coal which insignificantly inhibited the bacterial microbiota (e.g. *Streptomyces pyogenes, Pseudomonas aeruginosa*).

But, by simple changes regarding the initial chemical structure of the organic acids (by acylation), it is possible to increase their biological activity.

Generally, fulvic acids (FA) made favorable associations to soil sustainable characteristics, determining a better water penetration in compacted soils, root growth and development, increase organic matter content, root size, water retention, retain or leach plant nutrients.

(Canellas et al., 2002; Li et al., 2019; Dariellys et al., 2014; Just al., 2021).

The ascending chromatograms of the water-soluble fulvic sub-fraction of Mollic Gleysol revealed the qualitative structure and organizing of the organic compounds with low molecular weigh.

The organic material accumulations in the fulvic sub-fractions, exprime the potential of the microbiome to bio-synthesize secondary exometabolites, biosynthesis specific to each type of soil.

The chromatogram made possible to distinguish different characteristics, based on the specificity of the type of microbiome, as well as indications on the complexity and on an effect probable to making the conjugates by specific features to the ligand organic compounds, with characteristics induced by the microorganisms.

The biosynthesis of low molecular weight organic compounds, especially water-soluble, with specificity to each type of soil could be it considered effective becouse react with the minerals very quickly for the organic matter stabilization.

In subfraction of Mollic Gleysol (Figure 6), the elution chromatogram showed, after absorption of the extract of the fulvic subfractions on activated carbon, the accumulations of organic compounds soluble in acidified water, with relatively low molecular weights, such as carbohydrates and amino acids.

The structuring of the compounds of the fulvic sub-fraction appear on the background of a richer and more diversified organic matter.

Also, in sub-fraction, some accumulations of water-soluble organic compounds, such as polysaccharide compounds, mono-carbohydrates, amino sugars, were well highlighted in the case of soil.

After dialysis, the chromatogram revealed a colour content, formed mainly by colored compounds such as amino sugars, pentose, organic compounds with phosphorus, well highlighted the accumulations of organic compounds soluble in alkaline water, with relatively higher molecular weights.

The chromatogram of fulvic sub-fraction showed the structuring of it compounds, the formation of the short-acting and highly mobile compounds. The coloring suggest an intense biosynthesis of such mobile organic compounds.
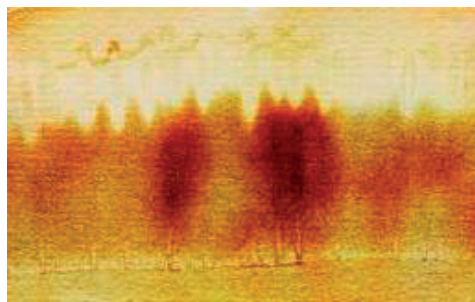

Figure 6. The image of ascending specific chromatogram of the alkaline water-soluble fulvic subfraction from Mollic Gleysol

The formation of complexes with inorganic constituents and the evolution to compounds with insoluble character indicate a intensification of association reactions. Thick migration fronts and dark external areas appear, if in the extract, the concentration of fulvic compounds is high. The fulvic sub-fraction show an increase of the mineral diversity in soil, relatively integrated in the organic material, structural complexity increases with the high level from microbial activity.The increase in intensity of microbial activities maintains the accumulation and integration of biosynthesized organic material.

Mainly, the stabilization of microbial metabolites on mineral surfaces influence the formation of stable organic matter in the soil (Li et al., 2019; Giannetta et al., 2019; Just et al., 2021; Kleber et al., 2021; Song et al., 2022). Organic compounds linked come mostly from microbial metabolism, appear layered on the clay particles and stabilized, due to the content in proteins and polysaccharides of the microbial residues.

In Figure 7 were presented the conjugates between water-soluble fulvic subfraction and siderophores from each strain of probiotic bacteria (LAB 41, LAB 57, LAB 62, LAB 69, LAB 83). The fluorescent highlight of the conjugates was proportional with chemical characteristics and specific production of siderophores.

The advantages offered by siderophore-fulvic subfraction conjugates include increased antifungal potency, improved selectivity for

phytopathogens, turning them into susceptible microorganisms. Perhaps such high molecular weight conjugates should be considered because large molecules can be tolerate, can be effective, relatively similar to the mode of action of natural antibiotics (Blaskovich et. al., 2015). In addition, it would be important to make more conjugates with structurally diverse natural siderophores as more than 500 siderophores have been reported (Hider & Kong, 2010). Possibly, such conjugates cannot be considered definitive solutions regarding the presence/persistence of phytopathogens, but important molecular structures in the fight, through their antimicrobial potential (Ouchetto et al., 2005; Noel et al., 2011).
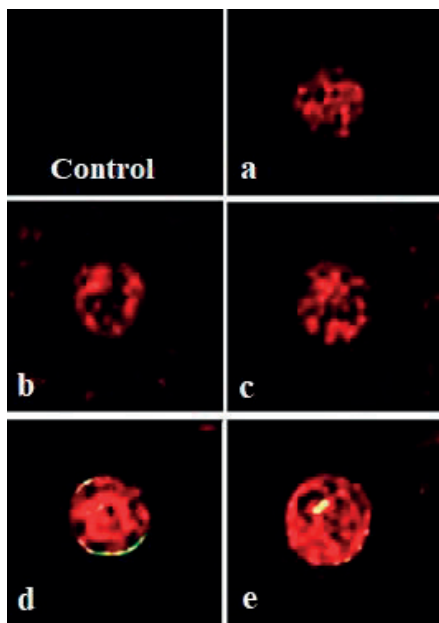
Figure 7. Fluorescent highlighting of conjugates between water-soluble fulvic subfraction and siderophores from a)LAB 41, b) LAB 57, c)LAB 62, d)LAB 69, e)LAB 83

After five days of incubation, the antifungal activity of the conjugates against phytopathogens (*P. expansum, A. ochraceus, A. flavus*) was evaluated by measuring the diameter of the inhibition zone around the wells. The agar plates were photographed and later analyzed. The inhibition zone was measured (mm) on the plates with the dispersed phytopathogens to estimate the antifungal activity. The inhibition of the phytopathogens analyzed was the lowest in the case of the bacterial strain LAB 57, the diameter of the inhibition zone being 11.6 mm for *P. expansum*, 12.4 mm for *A. flavus* and 12.7 mm for *A. ochraceus*. The strain LAB 83 determined the strongest inhibition of phytopathogens, the diameter of the inhibition zone being 24.5 mm for *P. expansum*, 22.3 mm for *A. flavus* and 20.2 mm for *A. ochraceus* The values of the inhibition zones determined for the other probiotic strains were intermediate (Figures 8).
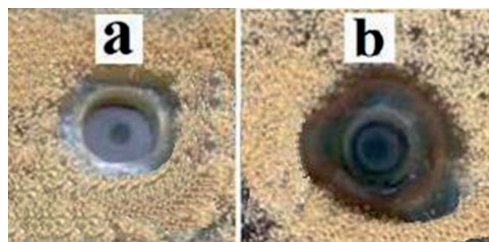
Figure 8. Inhibition zone of *A. ochraceus* induced by the conjugates between fulvic subfraction and siderophores from a) LAB 57, b) LAB 83

Probiotic bacteria possess plasma membrane-localized siderophores, consisting of ligands that form complexes that cover ferric ion coordination sites and achieve increased iron binding constants. For phytopathogens in different plant hosts, iron is no longer available, but tightly sequestered. Blocking access to iron by invading phytopathogens triggers the host's defense response by creating nutritional immunity (Nazarov et al., 2020). As a result, phytopathogens had to develop sophisticated strategies to ensure iron supply and the control of absorption can be considered a major homeostatic mechanism of them.

Probiotics directly intervene in the biocontrol of phytopathogens by modulating nutrient absorption (phosphorus and potassium), hormone production, nitrogen fixation and siderophores. Through the diversity of compounds with an antimicrobial role, probiotics also intervene indirectly in the biological control of phytopathogens by causing reactions to induce systemic resistance, mechanisms involved in the attack, as well as by decreasing the availability of iron for the phytopathogen (Sathe et al., 2007; Fhoula et al., 2013; Guo et al., 2013; Sangmanee and

Hongpattarakere, 2014; Cortes-Zavaleta et al., 2014; Mislin et al., 2014;Lamont et al., 2017, Kharazian et al., 2017; Juodeikiene et al., 2018; Arena et al., 2019; Sadiq et al., 2019; Muhialdin et al., 2020; Chen at al., 2021; Patel et al., 2021; Dopazo et al., 2022; Jaini et al., 2022).

The siderophores produced by the selected probiotic bacteria help plants to obtain iron requirements from the environment. Its absorption also acts antagonistically to phytopathogens in the soil by limiting its availability.

Studies have highlighted their inhibitory potential against phytopathogens, based on the ability to produce siderophores. Thus, bacterial probiotics show an antifungal effect against *Z. tritici* and the species *Lactiplantibacillus plantarum* exerts a strong antagonism against the necrotrophic fungus *Botrytis cinerea* (Lynch et al., 2016; De Simone et al., 2021).

Species of the *Lactobacillus* genus also show antifungal activity against *P. digitatum* and other phytopathogenic species (Matei et al., 2019).

Also, an antifungal effect of probiotic strain S2 filtrate against *Aspergillus flavus* was reported by affecting fungal growth, spore numbers (with two orders of magnitude), morphology, the level of aflatoxins produced (Al-Haik et al., 2017).

Due to the function of virulence determinants, probiotic bacteria can develop a diverse range of siderophore sequestering compounds, and in response, phytopathogens create mechanisms to avoid siderophore recognition by sequestering compounds (Sia et al., 2013; Koh et al., 2015).

The involvement of probiotic bacteria, as potentially useful agents against phytopathogens, results from the biosecurity elements and from the way of use in the biostimulation of some processes (Visser et al., 1986; Higa & Kinjo, 1989; Gajbhiye & Kapadnis, 2016; Daranas et al., 2019; Haryadi et al., 2019; Lim et al., 2019; Alexander et al., 2021; Duha & Abdullah, 2021; Malik et al., 2021;Abhyankar et al., 2022; Jaini et al., 2022). Thus, their GRAS status and history in food research make them ideal for use in biocontrol.

CONCLUSIONS

It was highlighted that the strains LAB 62, LAB 69 and LAB 83 produced siderophores in large quantities, under the established conditions, after an incubation period of 48 - 96 hours.

All five isolates produced catecholate-type siderophores, and of these, three strains also produced the hydroxymate type, under iron stress conditions.

The selected probiotic strains of *Lactobacillus* spp. produced siderophores in vary quantities.

Our isolates were shown to be potent siderophore-producing probiotic strains capable of forming conjugates with the water-soluble fulvic subfraction of Mollic Gleysol.

Siderophore-fulvic subfraction conjugates, through the effect of inhibiting phytopathogenic agents, make it possible to apply and increase the suppressive capacity of soils.

ACKNOWLEDGEMENTS

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