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Application of *Aureobasidium pullulans* in iron-poor soil. Can the production of siderophores improve iron bioavailability and yeast antagonistic activity?

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Abstract

Iron is a fundamental element for plants as well as for microorganisms and several pathogens, including *Rhizoctonia solani*, one of the main soil-borne pathogens of tomato. This study demonstrated the ability of *Aureobasidium pullulans* strain L1 to produce siderophores and how these molecules were, directly and indirectly, connected to its antagonistic activity and iron bioavailability. By ICP-OES (Inductively Coupled Plasma - Optical Emission Spectroscopy) trace analysis, the strain displayed the ability to increase the bioavailability of Fe (II) in the soil by almost 50% 30 days after inoculation. Also, the bioavailability of Mn, Cu, and Zn was increased after 30 days of incubation in the soil by 31.8%, 38.4%, and 27.1%, respectively. In *in vivo* assays, *A. pullulans* L1 strain showed a growth promotion of tomato roots length and stem diameter, respectively by 19.1% and 27.3%, and acted as a biocontrol agent (BCA) against *R. solani* (80% of inhibition). The results demonstrate a new aspect of this microorganism, usually applied as an antagonist of postharvest fruit diseases, to explore in different environments and against different pathogens, such as soil-borne pathogens.

Keywords

Yeast, siderophore, iron, tomato**,** soil-borne pathogen

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1 | Introduction

Iron is an essential micronutrient with various cellular functions, and its deficiency represents one of the most serious problems for plants nutrition. Nevertheless, plants have two major problems with iron as a free ion: its insolubility and its toxicity (Hell & Stephan, 2003). Iron is one of the most abundant metals in the earth's crust but its availability to plant roots is very low. For this reason, non-graminaceous plants reduce Fe^{3+} via a membrane-bound reductase to make it accessible for uptake by a Fe^{2+} transporter (Morrissey & Guerinot, 2009).

However, iron bioavailability can also depend on different uptake strategies amongst microorganisms (Hassler et al., 2012), such as siderophores production. Siderophores are low-molecular-weight, iron-chelating ligands produced by nearly all microorganisms because iron is an essential nutrient for all forms of life and is difficult to obtain due to its low solubility.

From an ecological point of view, *Aureobasidium pullulans* yeasts are ubiquitous species found mainly in soil, including Antarctic soils, water, phylloplane, wood, and many other plant materials, rocks, monuments, and limestone (Urzì et al., 1995; Gostinčar et al., 2014).

Also, it has been confirmed by Riquelme (1996) that yeasts produce only hydroxamate-type siderophores, while bacteria both hydroxamate and catecholate-type. Hydroxamate siderophores are derived from the non-proteinogenic amino acid ornithine and can be classified into four structural families: fusarinines, coprogens, ferrichromes, and rhodotorulic acid (Johnson, 2008).

Furthermore, hydroxamate siderophores exhibit extreme acid stability and this group of siderophores is the one most often found in nature (Saha et al., 2016; Złoch et al., 2016). These compounds have found different applications in the environment and agriculture (Kurth et al., 2016).

Recently, Di Francesco & Baraldi (2021a) showed that *A. pullulans* competes with *Monilinia laxa* (causal agent of stone fruits brown rot) to acquire iron by producing siderophore which reduces pathogen virulence. Also, different microorganisms were involved in metal recovery or remediation

of waste sites and polluted environments (Li et al., 2004) or promote the growth of plants via iron uptake (Vejan et al., 2016). Many bacteria associated with plants, e.g. rhizosphere and endophytic bacteria, can synthesize siderophores, which gives them a competitive advantage in the colonization of plant tissues by helping them to exclude other microorganisms from the same ecological niche (Hrynkiewicz et al., 2010; Loaces et al., 2011). Microbial siderophores can also contribute to the suppression of pathogens in the rhizosphere (Buyer & Leong, 1986). However, yeasts have received little attention as biocontrol agents (BCAs) of soil-borne fungal plant pathogens in comparison to bacterial, actinomycetes, and filamentous fungal antagonists (El-Tarabily & Sivasithamparam, 2006). *Aureobasidium pullulans* showed similar mechanisms of action against soil-borne pathogens such as *Rhizoctonia solani* and plant's aerial parts pathogens such as *Monilinia* spp. (Di Francesco et al., 2021a,b). Their ability to multiply rapidly, to produce antifungal diffusible metabolites, and cell wall-degrading enzymes (CWDEs) (Kohl et al., 2019), is a hallmark for potential application as soil BCAs and/or plant growth promoters (PGPs).

However, siderophore biosynthesis by microorganisms is directly connected with iron bioavailability in the soil. Eshaghi et al. (2019) found that great amounts of siderophores were synthesized in absence of iron whereas no siderophore production by bacteria was observed in the presence of a substantial amount of available iron. In addition to their primary function in iron mobilization, siderophores can chelate and release available to plants Al^{3+}, Zn^{2+}, Cu^{2+}, Pb^{2+}, and Cd^{2+} (Złoch et al., 2016; Kumar et al., 2018) and protect microbes against metal toxicity (Cortese et al., 2002; Fekete & Barton, 1992). Considering these facts, we performed a study in which we aimed at (i) confirming the ability of *A. pullulans* strain L1 to produce siderophores by *in vitro* assays; (ii) evaluating the ability of the L1 strain to improve iron availability in the soil with a low iron content; (iii) verifying its role as a PGP and (iv) as an antagonist of *R. solani* soil-borne pathogen through the iron competition.

2 | Materials and methods

2.1 | Microorganisms

Aureobasidium pullulans L1 strain (Di Francesco et al., 2018a) was obtained from the fungal collection of Criof (Department of Agricultural Sciences, University of Bologna, Italy). The strain was maintained on nutrient yeast dextrose agar (NYDA: 8 g of nutrient broth, 5 g of yeast extract, 10 g of dextrose, and 15 g of technical agar (Oxoid, Basingstoke, UK) in 1 L of distilled water and stored at 4°C until use. Two days before the experiments, the antagonist was grown on NYDA at 25°C, and yeast cells were collected in sterile distilled water containing 0.05% (v/v) Tween80 (Sigma-Aldrich, St. Louis, MO, USA) and quantified for a final concentration of 10^8 cells mL^{-1} by counting spore suspension on a hemocytometer cell. The final concentration was adjusted with sterile distilled water.

Rhizoctonia solani isolate (Rs1) derived from Criof fungal collection (Di Francesco et al., 2021b). For *in vitro* experiments, the isolate was maintained on Potato Dextrose Agar (PDA, 39 g in 1 L of distilled water, Oxoid, Basingstoke, UK) at 25°C for 5 days until use. For *in vivo* experiments, the inoculum for infecting plants was prepared by cutting plugs (6 mm of diameter) from a fungal colony grown 5 days, which was then transferred to conical flasks containing 50 mL of Potato Dextrose Broth (PDB, 39 g in 1 L of distilled water, Oxoid, Basingstoke, UK). The inoculated flasks were cultured at 25°C in a rotary shaker (250 rpm) for 5 days. The culture was centrifuged at 5000×$\boldsymbol{g}$ for 20 min at 4°C and the mycelium was washed with distilled water, filtered through two layers of miracloth, finally homogenized by using a mixer (Imetec Ch4, Italy) and immediately used.

2.2 | Soil and plant

The target soil was taken from a parcel of Bologna University's experimental fields (Cadriano, Bologna, Italy). It was analyzed according to the SSSA (Soil Science Society of America) methods for soil chemical analysis (Sparks et al., 1996).

The soil (*Udic Ustochreps* according to USDA soil taxonomy) was clayey-sandy (36% sand, 38% silt, 26% clay), with sub-acid pH (6.5), water holding capacity of 28.3%, with low nutrient content, having total organic carbon of 0.66%, total nitrogen of 0.08% and bioavailable Fe content (EDTA extractable) of 13.2 mg kg^{-1}. The soil was exposed to oven drying at 70°C for 48 h, grounded, and sieved by a 2 mm mesh sieve.

Tomato (*Solanum lycopersicum* L.) plants were obtained from seeds cv 'Datterini' (Syngenta, Switzerland) seeded in 50 mL pots filled with moss until the 2 expanded leaves stage (15 days) under daylight conditions at 25 ± 2°C and 60% of relative humidity (RH).

2.3 | *In vitro* assay: spectrophotometric determination of iron reduction activity by L1 strain

Sterile flasks containing 100 mL of liquid Minimal Medium (MM: 30 g of sucrose, 2 g of $NaNO_3$, 1 g of KH_2PO_4, 0.5 g of $MgSO_4*7H_2O$, 0.5 of KCl, 0.2 mL of sterile mineral solution in 1 L of distilled water) were amended by different $FeCl_3*6H_2O$ (Sigma-Aldrich) concentrations (20, 50, 100, 500 mg L^{-1}), 168 mg of L-ornithine (Sigma-Aldrich) (as described by Wang et al., 2009a; Di Francesco & Baraldi, 2021a), and 10 mL of *A. pullulans* L1 strain (10^8 cells mL^{-1}) suspension. Flasks were incubated at 25°C in a rotary shaker (250 rpm) for 28 days. Controls were represented by flasks containing MM amended with L-ornithine and iron concentrations as above-described without yeast, and MM amended only with L-ornithine and L1 suspension. The sample unit consisted of five flasks (replicates). After 1 h and each 7 days thereafter until the 28^{th} days of incubation, aliquots of 2 mL from each flask were collected and analyzed to determine Fe (II) and Fe (III) content through the method described by Tamura et al. (1974), with some modifications. For the determination of Fe (II) concentration, an aliquot of 0.5 mL of each sample was diluted with 4 mL of distilled water and treated with 0.1 mL of hydroxylamine solution (100 g L^{-1} in water), followed by heating in boiling water for 10 min. After cooling at room temperature, the solution was treated with 2 mL of acetate buffer pH 5.0 (10%), 1 mL o-phenanthroline solution (0.1%), 2 mL of distilled water, and then subjected to spectrophotometric analysis at a wavelength of 510 nm.

For the overall determination of Fe (II) and Fe (III), the same method was followed, with a further step, by adding 0.1 mL of 6 M HCl to the sample before boiling, to reduce Fe (III) to Fe (II). In this way, the concentration of Fe (III) was calculated as the difference between the second value (Fe II + Fe III) and the first one (Fe II). The assay was repeated twice.

2.4 | Iron chelating activity by L1 strain: determination of available iron in the soil through optical emission spectroscopy (ICP-OES)

Plastic pots were filled with 250 g of dry soil, soaked with distilled water (50% of field capacity), weighed, and pre-incubated at 20°C for 2 weeks, keeping the humidity constant by adding distilled water (if necessary). Three different concentrations of $FeCl_3*6H_2O$ (Sigma-Aldrich) (0, 100, and 400 mg kg^{-1}) and 10 mL of the L1 strain suspension (10^8 cells mL^{-1}) were added to the soil. Controls with no yeast were amended with the iron concentrations mentioned above and 10 mL of distilled water. The sample unit consisted of three jars (replicates) for the controls and each iron concentration.

Soils were incubated at 20°C for 90 days. Jars humidity was controlled each 7 days by weighing and maintained stable adding distilled water if necessary. After 1 h, 15, 30, 60, and 90 days, 11 g of wet soil, corresponding to 10 g of dry soil, were collected from each pot. Bioavailable metals were extracted as described in Lakanen (1971), by adding to each sample 50 mL of a 0.02 M EDTA/0.5 M ammonium acetate solution at pH 4.65, shaking at room temperature for 30 min at 120 rpm, and finally filtering through a filter paper (Whatman no. 42, 125 mm of diameter). The determination of bioavailable elements was carried out by an inductively coupled plasma optical emission spectrometer (ICP-OES, Arcos, Ametek Spectro) (Mallampati et al., 2013).

2.5 | L1 population dynamic

To study the population dynamic of the L1 strain in the soil, antagonist cells were collected 1 h, 15, 30, 60, and 90 days after the treatment. The concentration of yeast cells was determined as

described by Di Francesco et al. (2018b) with some modifications. One g of soil was transferred into a sterile stomacher bag containing 10 mL of sterile distilled water and Tween 80 (0.05%). The bag was stomached for 10 min (Bag Mixer 400; Interscience, St Nom, France). The resulting slurry, with prior dilution in sterile distilled water, was surface plated on NYDA. Petri dishes were incubated at 20°C for 2 days. The sample unit consisted of five plates for each sample soil treatment and the experiment was performed twice.

2.6 | *In vivo* assays

In vivo experiments were conducted to verify the effectiveness of the L1 strain to increase soil iron bioavailability, tomato plant growth promotion, and *R. solani* virulence reduction. Tomato plants were transplanted in new plots containing 220 g of the above-described soil (1 plant per pot) amended with 100 mg kg^{-1} of $FeCl_3*6H_2O$ (Sigma-Aldrich), 10 mL of the L1 strain suspension (10^8 cells mL^{-1}), and 1 g of *R. solani*. The control consisted of soil amended with water only. The sample unit consisted of 10 plants (replicates) for each treatment. The assay was conducted twice. After the stem-end symptom appearance (1 month) the disease incidence was evaluated, and Koch postulates were conducted. Plants hypocotyls with severity of *R. solani* were evaluated by using a 0–4 scale developed by Cardoso & Echandi (1987) with some modifications, where: 0 = no lesions, 1 = lesions < 2.5 mm, 2 = lesions 2.5–5 mm, 3 = lesions > 5 mm, 4 = lesions girdling plant and wilting visible on leaves. The disease severity index (DSI) was calculated for each treatment using the formula:

$$DSI = \frac{percentage\ of\ incidence * mean\ severity}{5}$$

To verify the PGP ability of the L1 strain on tomato plants, the length of the roots (mm), the stems diameter (mm), and the leaves areas (mm^2) were measured after 1 month from the treatment by using a digital caliber (Dirtygal) and Tomato Analyzer Software (Brewer et al., 2006), respectively.

Treatment effects were assayed in two independent experiments and the control consisted of soil treated with water only. The sample unit consisted of ten plants (replicates) for each treatment. Samples of soil, from pots containing tomato plants and the treatments, were collected, and analyzed as described in paragraph 2.5.

2.7 | Statistical analysis

Data were analysed by one-way ANOVA. Separation of means was performed using the least significant difference (LSD) test at $P < 0.05$. All analyses were performed with the software Statgraphic Plus Version 2.1 (Statistical Graphics Corp., USA 1996).

3 | Results

3.1 | *In vitro* assay: iron reduction activity by L1 strain

This assay was conducted to evaluate the capability of the strain L1 to take iron (III) and ensure the iron (II) bioavailability. The culture medium was amended with $FeCl_3*6H_2O$ (Sigma-Aldrich) concentrations (20, 50, 100, and 500 mg L^{-1}).

The strain L1, in presence of 20 mg L^{-1} of iron (III), after 21 days of incubation, displayed a consumption of the Fe (III) by 50%, releasing 14 mg L^{-1} of Fe (II) in the culture medium. Also, in presence of 50 and 100 mg L^{-1} of iron (III), after 21 days of incubation, the yeast strain released the highest content of the Fe (II), respectively of 12 and 24 mg L^{-1}. The highest initial concentration of Fe (III) (500 mg L^{-1}) did not cause an increase of bioavailable iron, on the contrary, it seemed to inhibit yeast strain metabolism (Table 1).

3.2 | Iron chelating activity by *Aureobasidium pullulans* L1 strain in the soil

The experiment was conducted to verify the capability of L1 strain in the soil to chelate Fe (III) and consequently increasing its bioavailability.

From the ICP-OES analysis, results showed an inverse correlation between the presence of the initial highest Fe concentration (400 mg kg^{-1}) in the soil and the iron reduction activity displayed by the yeast strain (Fig. 1). With 0 mg kg^{-1} of Fe, the bioavailability of the metal increased by almost 50% after 30 days from the yeast inoculation. The addition of 100 mg kg^{-1} of Fe (III) in the soil increased the bioavailability of the metal after 30 days by only 9.9% through the reduction activity exerted by the L1 strain. The highest Fe concentration in the soil reduced significantly in time the yeast strain metabolism. By ICP-OES, the ability of the L1 strain to increase the bioavailability of other metals such as Cu, Zn, and Mn, after 30 days in the soil, was also detected (Table 2 and Fig. 2).

Mn was significantly increased by the L1 strain by 3.7% and 31.8% respectively after 15 and 30 days (Table 2). Cu and Zn bioavailability was increased by the L1 strain after 30 days by 38.4% and 27.1%, respectively (Fig. 2).

3.3 | Dynamic population of L1 strain in the soil

Simultaneously with the spectrophotometric analysis conducted with ICP-OES, the growth of the L1 strain in the soil was verified in the presence of different concentrations of Fe (III) (0, 100, and 400 mg kg^{-1}). The data showed that the strain exponential growth in the first 15 days from the initial inoculation was greater with 0 mg kg^{-1} and 100 mg kg^{-1} of Fe (III) with a log phase of 3.6, concerning the soil containing the highest concentration of Fe (log 3.4) (Fig. 3). In the subsequent time intervals, the behaviour of the microorganism shows two different trends (Fig. 3). At the lowest iron concentrations (0 and 100 mg kg^{-1}), L1 determines a decrease in its growth at 30 days, in correspondence with the highest bioavailability of the target metal (as reported in the previous paragraph). The strain reached its maximum growth after 60 days, mainly in the soil without Fe addition (log 3.8), to decrease in time.

At the highest concentration (400 mg kg^{-1}) after 15 days, the L1 growth remained stationary, probably related to the physiological inhibitory effect of the high concentration of Fe (III) on the yeast strain.

3.4 | *In vivo* assays

The experiments were conducted to verify the ability of the L1 strain to improve the tomato plant's growth in poor soil concerning its capability to increase the iron bioavailability and to act against *R. solani* as a BCA**.** The diameter of the stem, the roots length, and the leaf area were taken as parameters to evaluate the L1 strain biostimulant efficacy.

The length of the roots increased only in presence of L1 by 43.8%, concerning the control. In the soil amended with Fe (III) no evident variations were detected, both in the presence and absence of L1, but an increase in terms of root length was detected in comparison to the control (13.5% and 19.1, respectively). As for the stem diameter, the presence of the L1 strain in the soil, both in the absence and in the presence of 100 mg kg^{-1} of Fe (III), showed an increase by 18.1% and 27.3%, respectively (Table 3).

Regarding the leaf area, plants grown in the soil treated only with L1 showed an increase by 47% with respect to the control (Table 3). However, in the case of plants grown on soil treated with Fe (100 mg kg^{-1}) and L1 strain, a not significant increase was observed (Table 3).

Regarding the L1 strain antagonistic behavior against *R. solani*, the unique presence of the yeast reduced the disease incidence in the plant and by 80% in presence of 100 mg kg^{-1} of iron, concerning to the relative control. However, the severity disease index was relatively low (Table 4), probably due to the poor soil composition. These results were probably correlated to those of the diameter of the stem and the length of the roots showed in Table 4, where plants grown in presence of L1 showed an increase by 52.6% and 46.5%, and by 19.2% and 33.3% with Fe (III) respectively, compared to the relative controls.

Furthermore, it is observed that the infected plants in the presence of L1 in the soil showed a higher value of leaf area (+245.4%, +200%) concerning to the controls (Table 4).

After 30 days, in the soil with the plant, L1 growth increased by 48% concerning to the starting inoculation. With Fe (III) and *R. solani*, its growth increase was by 44% and 32% respectively (data not shown).

4 | Discussion

Iron deficiency is a worldwide agricultural problem, with major implications in crop production. The most common practice to overcome this problem is the application of synthetic iron chelates (Nadal et al., 2012), however, they usually are not biodegradable (Bucheli-Witschel & Egli, 2001). Fertilization with synthetic chelates is the most effective agricultural practice to prevent iron deficiencies especially for cash crops in calcareous soils. At the same time, these compounds are persistent in the environment and subject to the risk of metal leaching from the soils. Consequently, their accumulation in the environment is becoming a matter of great concern (Bucheli-Witschel & Egli, 2001). The search for better, cheaper, and more environmental-friendly alternatives represents a major challenge. The use of antagonist and PGP microorganisms, can be an alternative proposal for more sustainable and environmental-friendly development of agriculture, since fungicides have the potential to contaminate water and soil (Kaonga et al., 2017). Microorganisms have the potential to increase plant nutrient bioavailability through nitrogen fixation and mobilization of phosphorus, potassium, and iron to the crop plants (Rashid et al., 2016). In such circumstances, some bacterial and fungal strains (Sharma et al., 2003; Vansuyt et al., 2007) synthesized low-molecular-mass proteins known as siderophores with a high affinity to chelate (Machuca et al., 2007; Miethke & Marahiel, 2007) and solubilize iron from mineral or organic compounds. Di Francesco et al. (2021a,b) verified the ability of *A. pullulans* to produce siderophores and how these molecules were, directly and indirectly, connected respectively to its

antagonistic and PGP behaviour. The antagonist displayed the ability to increase the bioavailability of Fe (II) starting from 7 days of incubation in a medium amended with 20 mg L^{-1} of Fe (III) until 21 days. In presence of higher concentrations of Fe (III) (50 and 100 mg L^{-1}), the strain L1 after 21 days of incubation released more bioavailable iron. Conversely, in presence of 500 mg L^{-1}, the microorganism ceases any metabolic activity. Iron is an essential nutrient for yeasts, although toxic when present at higher concentrations in growth media (Gaensly et al., 2015). For this reason, iron uptake and utilization by yeasts are tightly controlled (Askwith et al., 1996). For example, *Saccharomyces cerevisiae* can easily thrive in environments when the iron is too scarce or too abundant, which suggests it responds to iron depletion by altering its utilization, preserving this nutrient in essential metabolic pathways (Philpott et al., 2008). Determining the optimal concentration of a microelement in the culture media as well as the concentration in which it will inhibit yeast biomass formation is a complex process that depends on the employed microorganism and on the culture media composition.

Also, against *M. laxa*, the highest reduction results were revealed by *A. pullulans* strains in presence of lower iron concentrations, confirming that the microorganism displayed its antagonistic efficacy when exogenous nutrients were scarce (Sanz Ferramola et al., 2013). Plante & Labbè (2019) showed how under low iron conditions siderophore synthetase expression of *Schizosaccaromyces pombe* remains upregulated. Even in the case of the pathogen, low or/and high iron conditions, negatively influenced its virulence, probably because the plant host might have evolved mechanisms to sequester iron from pathogens during the infection process (Herlihy et al., 2020). This fact justified our results, wherewith soil iron addition *R. solani* was less aggressive concerning to the iron absence condition.

According to previous observations on nutritional requirements of *Monilinia* spp. (Byrde & Willetts, 1977), the conidial germination rate of the pathogen resulted low when grown in minimal medium. This confirmed that the target pathogen is nutrient-dependent and needs an adequate

quantity of supplements for its growth: both conidial germination and hyphal development (Bencheqroun et al., 2006; Di Francesco et al., 2017).

Regarding to the soil, which could be a suitable environment for the exploitation of this yeast, L1 showed the capability to increase iron bioavailability only after 30 days of incubation, without and with 100 mg kg^{-1} of Fe (III) addition. Also, bioavailable Cu and Mn were increased in the soil after 30 days by L1 strain metabolism activity. Availability of Fe, Cu, and Mn could be directly associated with yeast's exponential growth phase. Metal availability increased after 30 d, in correspondence of that the growth strain slowed down, maybe due to the effect of metals in the soil. In confirmation of this, as shown by Bencheqroun et al. (2007), at higher concentrations of nutrients, a reduction of the activity of the microorganism was observed. As is well known, *A. pullulans* L1 strain possess antagonistic properties, able to severely reduce plant diseases incidence caused by fungal pathogens (Di Francesco et al., 2018a). Recently, Di Francesco et al. (2021b) discovered an indirect capability of *A. pullulans* to promote the growth of soybean and bean plants. The L1 strain can achieve these effects by several mechanisms: production of hydrolytic enzymes (Di Francesco et al., 2020), competition for nutrients (Di Francesco et al., 2018b), production of siderophores and antifungal compounds (Di Francesco et al., 2020, 2021a,b). In the present study, the L1 strain activity significantly increased the growth of tomato plant's roots, stem diameter, and leaf area more than with the addition of a soil improver (Fe^{3+}).

In line with what has been discovered and taking into consideration that the ability to produce siderophores of this species of yeast determines greater competitiveness towards some pathogens (Wachowska & Borowska, 2014; Wang et al., 2009a,b), its antagonism has been tested against the soil-borne pathogen *R. solani*, thus showing effectiveness in reducing fungal incidence in tomato plants. The obtained results showed that with the L1 strain and iron addition (100 mg kg^{-1}) in the soil, *R. solani* incidence was significantly reduced, and an increase of roots length and leaf area was determined. Furthermore, in this study, the pathogen isolate resulted less virulent with respect to a previous study (Di Francesco et al., 2021b) conducted in a soil with optimal conditions. This fact is

probably due to the lack of nutrients in the soil (Rosinger et al., 2019). The use of this strain to improve the soil iron bioavailability represents a novelty, because it was mainly used as a BCA of various fruit postharvest pathogens (Mari et al., 2012; Rusin et al., 2019).

Also, its ability to produce siderophores and increase iron bioavailability, indirectly stimulated tomato plant growth, representing an innovative aspect as PGP microorganism (Sun et al., 2019; Rajkumar et al., 2012). A further identified aspect of the tested strain, probably linked to the production of siderophores, was represented by the capability to alter the bioavailability of other nutrients such as Cu, Zn, and Mn, necessary for plant nutrition (Scarponi, 2003; Sequi, 2005). This nutrients improvement by L1 was recorded in the soil, as for iron, after 30 days of growth, without any further metal addition. Govarthan et al. (2016) showed that siderophores production by *Paenibacillus* sp. exerted a decrease of heavy metals content in soils, displaying a bio-remediation aspect (Abbaszadeh-Dahaji et al., 2019; Jinal et al., 2019). This study showed that *A. pullulans* L1 strain can produce siderophores and this characteristic can lead it to further applications given the traits that unite it to PGPs, in addition to those already recognized as antagonists of plant pathogens. For these reasons, this peculiarity could be interesting to investigate in different environments and against different soil-borne pathogens.

Conflict of Interest

The authors declare that they have no conflict of interest.

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FIGURES

Figure 1. Bioavailable Fe in the soil amended or not with Fe (III) concentrations (0, 100, 400 mg kg^{-1}) and L1 yeast strain suspension (10^8 cells mL^{-1}) after 0, 15, 30, 60, and 90 days of incubation at room temperature. Each value is the mean of three replicates. Same letters (lower case – comparison of the different treatments at the same time; capital letter – comparison of the same treatment in time) are not statistically significant according to LSD test ($P < 0.05$).

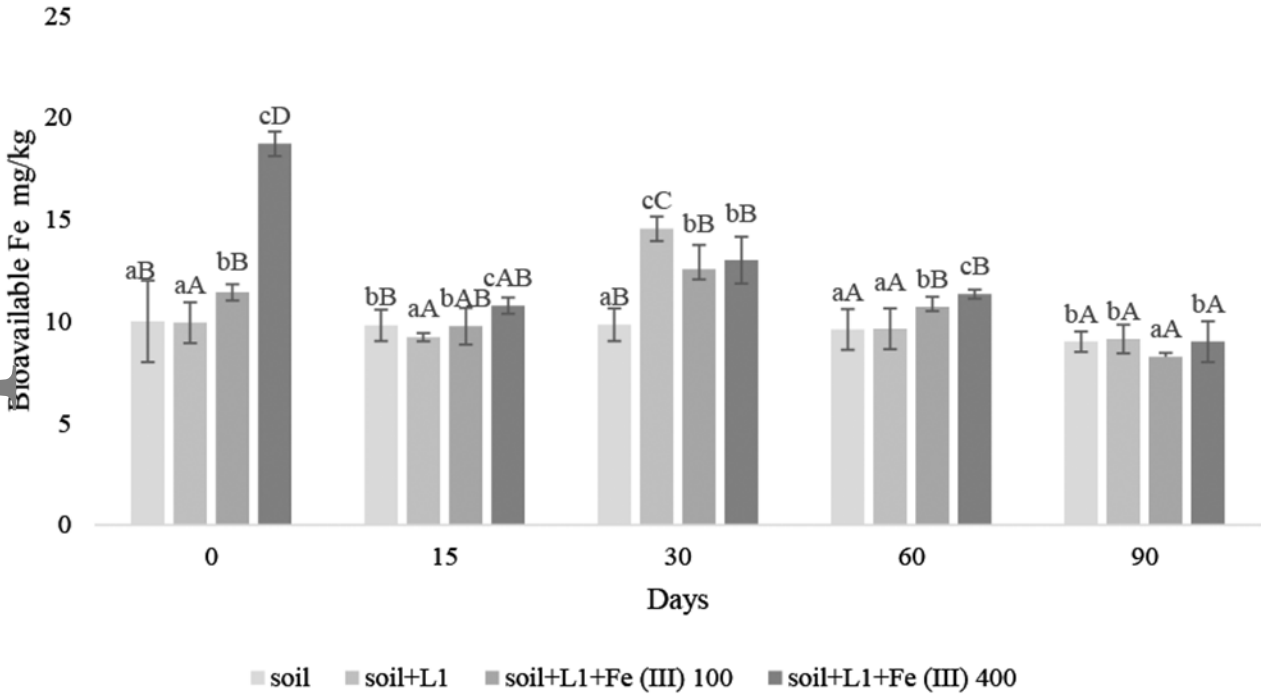

Figure 2. Copper (Cu) and Zinc (Zn) concentrations (mg/kg) in the soil after 0, 15, 30, 60, and 90 days of incubation with or not L1 yeast strain suspension (10^8 cells mL^{-1}) at room temperature. Each value is the mean of three replicates. Same letters within the comparison of the different treatments at the same time are not statistically significant according to LSD test ($P < 0.05$).

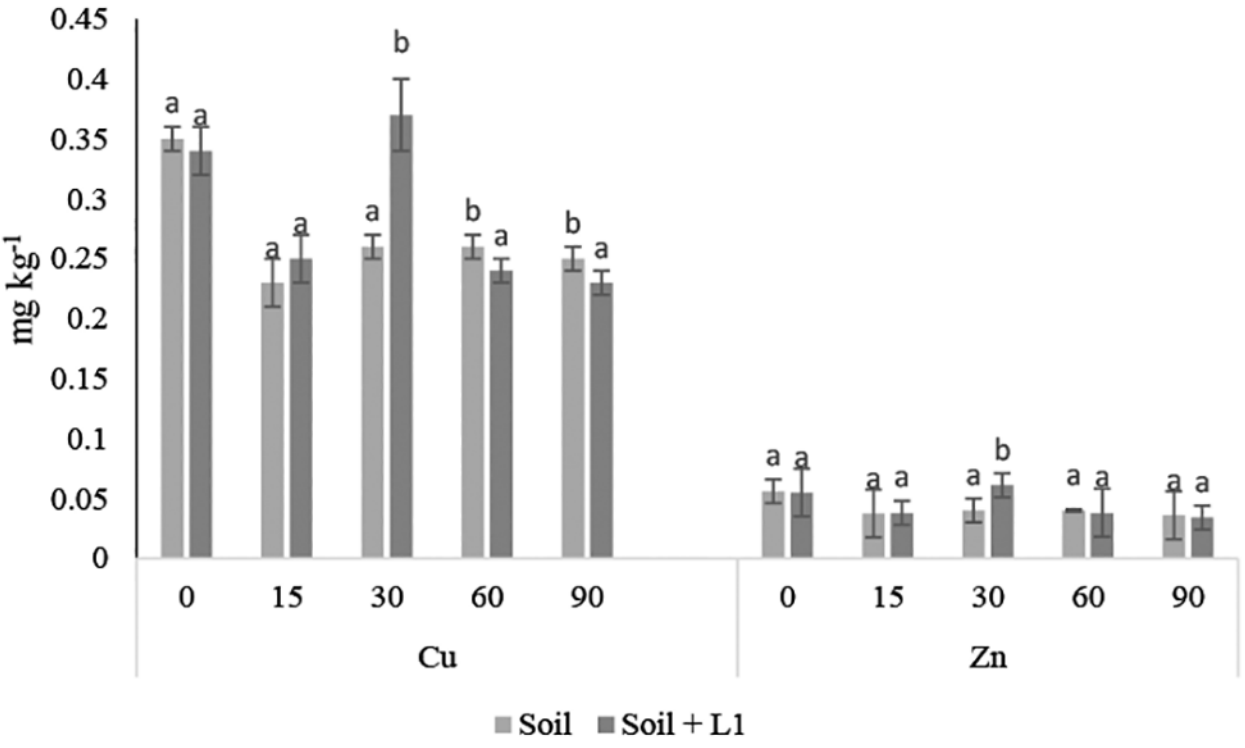

Figure 3. Population dynamics of *Aureobasidium pullulans* (L1 strain) in the soil after 0, 15, 30, 60, and 90 days of incubation at room temperature. Each point represents the mean of the number of colonies forming unit (CFU) from three replicates (soil samples), each plated five at each sampling time. Data are the means of two independent experiments ± standard error. Within the same treatment the asterisk indicates significant differences according to LSD test ($P < 0.05$).

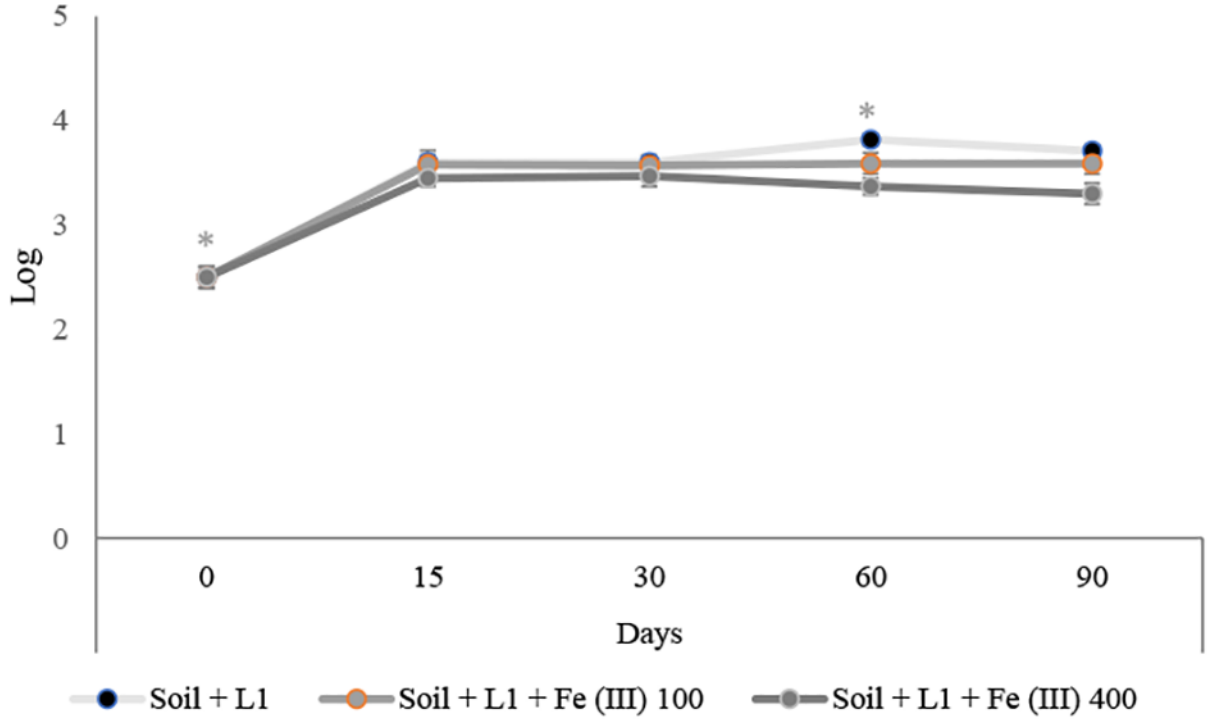

Table 1.

Influence of L1 strain on Fe (II) content after 0 (1 h), 7, 14, 21, and 28 days in Minimal Medium amended with 20, 50, 100, 500 mg/L of Fe (III) at 25°C. Each value is the mean of three replicates. Different letters within the same Fe (III) concentration indicate significant differences according to LSD test ($P < 0.05$).

	Fe (II) mg/L				
	Days				
Fe (III) Initial concentration	0	7	14	21	28
20 mg L^{-1}	2.2±0.7a	8.0±1.0b	9.3±0.4c	14±1.5d	2.6±0.5a
50 mg L^{-1}	2.1±0.3a	2.1±0.2a	2.0±0.2a	12±1.8c	10±1.1b
100 mg L^{-1}	4.8±0.3b	7.0±1.1c	11.3±1.5d	24.3±3.2e	2.6±0.6a
500 mg L^{-1}	14±3.6b	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a

Table 2.

Manganese (Mn) concentration (mg/kg) in the soil after 0, 15, 30, 60, and 90 days of incubation with or not L1 yeast strain suspension (10^8 cells mL^{-1}) at room temperature. Each value is the mean of three replicates. Same letters within the comparison of the different treatments at the same time are not statistically significant according to LSD test ($P < 0.05$).

	Mn mg/kg				
	Days				
	0	15	30	60	90
Treatment					
Soil	13±1.2a	10.8±2.1a	11±2.0a	10.6±1.9a	11±2.1a
Soil + L1	13.2±1.0a	11.5±1.8b	14.5±1.6b	10.7±1.7a	10.9±1.9a

Table 3.

Tomato plant roots length (mm), stem-end diameter (mm) and leaf area (cm^2) after one month from the treatment with L1 strain (10^8 cells mL^{-1}) and $FeCl_3*6H_2O$ (100 mg kg^{-1}). The control consisted of roots plants treated with water only. Each value is the mean of 10 replicates ± standard deviation Different letters within the same measurement indicate significant differences according to LSD test ($P < 0.05$).

		mm		cm^2
		Root length	Stem diameter	Leaf area
Solanum lycopersicum L.	Soil	162.16±15.6c	2.75±0.8b	4.00±1.6a
	Soil+L1	233.33±12.3d	3.25±0.3c	5.88±1.7b
	Soil+Fe (III)	131.66±10.4a	2.16±0.4a	4.03±1.3a
	Soil + Fe (III)+L1	140.00±6.9b	2.75±0.7b	4.33±2.1a

Table 4.

Effect of L1 *Aureobasidium pullulans* strains (10 ml, 10^8 cell mL^{-1}) and $FeCl_3*6H_2O$ (100 mg kg^{-1}) on disease incidence (DI), disease severity (DSI = disease severity index) at hypocotile, caused by *Rhizoctonia solani* (*Rs1*), and on roots length (mm), stem diameter mm), leaf area (cm^2) in presence of the soil borne pathogen. Tomato plants were transplanted in new plots containing 220 g of soil (1 plant per pot) amended with $FeCl_3*6H_2O$, L1 strain suspension, and *R. solani* (1 g). After the stem-end symptom appearance (one month) all the parameters were evaluated.

	Treatments	DI*	DSI**	Root lenght (mm)	Stem diameter (mm)	Leaf area (cm^2)
Solanum lycopersicum L.	Soil	40±1.50d	8	116.3±a	1.92±0.4a	1.10±0.3a
	Soil + L1	0.0±0.0a	0	170.3±c	2.90±0.3c	3.80±0.7c
	Soil + Fe (III)	25±0.44c	8	117.1±a	1.92±0.3a	1.19±0.3a
	Soil + L1+ Fe (III)	5.0±0.2b	2	156.8±b	2.29±0.2b	3.33±0.4b

Means (± SD) followed by the same letter in a column are not significantly different according to LSD test ($P < 0.05$)

* DI = disease incidence. Values are the mean of the two experiments ± standard deviation

* *Disease severity index (DSI) of tomato was calculated by percentage of disease incidence × mean of severity/5, where severity was rated on a 0–5 scale