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Effect of the endophytic plant growth promoting *Enterobacter ludwigii* EB4B on tomato growth

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Summary This study aims to develop a biocontrol agent against *Fusarium oxysporum* f.sp. *radicis-lycopersic*i (FORL) in tomato. For this, a set of 23 bacterial endophytic isolates has been screened for their ability to inhibit *in vitro* the growth of FORL using the dual plate assay. Three isolates with the most sound antagonistic activity to FORL have been qualitatively screened for siderophore production, phosphates solubilization and indolic acetic acid (IAA) synthesis as growth promotion traits. Antagonistic values of the three candidates against FORL were respectively: 51.51 % (EB4B), 51.18 % (EB22K) and 41.40 % (EB2A). Based on 16S rRNA gene sequence analysis, the isolates EB4B and EB22K were closely related to *Enterobacter ludwigii* EN-119, while the strain EB2A has been assigned to *Leclercia adecarboxylata* NBRC 102595. The promotion of tomato growth has been assessed *in vitro* using the strains EB2A, EB4B and EB22K in presence of the phytopathogen FORL. The treatments with the selected isolates increased significantly the root length and dry weight. Best results were observed in isolate EB4B in terms of growth promotion in the absence of FORL, improving 326.60 % of the root length and 142.70 % of plant dry weight if compared with untreated controls. In the presence of FORL, the strain EB4B improved both root length (180.81 %) and plant dry weight (202.15 %). These results encourage further characterization of the observed beneficial effect of *Enterobacter* sp. EB4B for a possible use as biofertilizer and biocontrol agent against FORL.

Additional keywords: Biocontrol, biofertilizer, *Enterobacter ludwigii*, PGPR

Introduction

The rhizospheric zone is rich in nutrients compared with the neighboring bulk soil due to the accumulation of a variety of organic compounds released by the roots through exudation, secretion and rhizodeposition. These organic compounds can be used as carbon and energy sources by microorganisms and microbial activity is particularly intense in the rhizosphere (Chauhan *et al*., 2015).

An alternative to increasing agricultural productivity in a sustainable way is the manipulation of micro-organisms that benefit the soil and plant health (Kloepper *et al*., 1989). For decades, rhizobacteria beneficial to plants are often referred to as plant growth promoting rhizobacteria (PGPR), which are characterized by at least two of the three following criteria: competitively root colonization, stimulation of growth and reduction of disease incidence (Reddy, 2013). Microbial-inoculants are being widely used to improve plant growth under controlled as well as natural field conditions (Nadeem *et al*., 2013).

Inoculants of PGPR bacteria improve root development through the production of certain phytohormones (Bloemberg and Lugtenberg, 2001), such as auxins including indole acetic acid (AIA), cytokinins and gibberellins (Vessey, 2003). Furthermore, many bacterial strains are able to improve the health of plants by limiting the saprophytic growth of phytopathogenic microorganisms, and some of them are used as biological control agents in agriculture (Bloemberg and Lugtenberg, 2001; Whipps, 2001). The great diversity of the mechanisms of action

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of these microorganisms is mainly related to their great ability to produce a wide range of secondary metabolites and to induce ISR in the host, making it less susceptible to subsequent infection by a pathogen (Van Loon, 2007; Weller *et al*., 2002).

PGPR include several bacterial endophytes with alleged positive effects on plant health and growth, which have been pursued mainly for agricultural applications to increase yields since three decades ago (Piccoli and Bottini, 2013). Endophytic bacterial strains selected on the basis of plant growth-promoting bacteria (PGPB) characteristics are useful for formulation of inoculants to improve growth and yield (Forchetti *et al*., 2010). Plant growth-promoting bacterial endophytes have been successfully used to induce fungal resistance in plants (Ji *et al*., 2014), and they are widely used in the developing areas of forest regeneration and phytoremediation of contaminated soils (Ryan *et al*., 2008).

PGPR modify the rhizospheric environment by producing antagonistic molecules with antibiotic or antifungal properties, or by synthesizing cell walls degrading enzymes and volatile organic compounds (VOC), which act against pathogens, disrupting bacterial cell–cell communication (*quorum sensing*) (Grobelak *et al*., 2015). In addition, many of these rhizobacterial strains can also improve plant tolerance against salinity, drought, flooding and heavy metal toxicity. Therefore, they enable plants to survive under unfavorable environmental conditions (Ma *et al*., 2011). Numerous works have reported beneficial effects of these rhizobacteria for improving plant growth under normal as well as stressful environment (Szepesi *et al*., 2009; Heidari and Golpayegani, 2012).

PGPR can additionally help plants by increasing their uptake of nutrient elements such as Phosphate (P). The low availability of this macronutrient to plants results from the fact that the P exists in insoluble forms (Kisiel and Kepczynska, 2016). Microbial phosphate solubilization is likely to be involved in a better plant growth, yield and nutrient uptake. Thus such trait is considered as a prospective tool for development of biofertilizers (Dey *et al*., 2004).

Siderophores are low molecular weight peptides or iron chelators (Santos-Villalobos *et al*., 2012). As soon as the complex siderophore-iron enters the cytosol the cells through specific siderophore receptors present in the cell membrane, the ferric iron gets reduced to a ferrous form which becomes free from the siderophore chelator complex. The released ferrous iron form is further used for metabolic processes (Venkat *et al*., 2017).

IAA is a secondary metabolite produced during the later stages of growth, after the stationary growth phase (Gupta *et al*., 2012), a phytohormone controlling many important physiological processes in plants such as cell division, tissue differentiation and root initiation (Khan *et al*., 2014).

Tomato, *Solanum lycopersicum* L. (Solanaceae) has been considered as one of the most important horticultural crop worldwide (Vos *et al*., 2014). However, tomato production has shown limitations arising from the use of cultivars susceptible to diseases and pests causing substantial production losses (Dias *et al*., 2017). Use of biological control agents, such as plant growth promoting rhizobacteria, can be a suitable approach in control of tomato diseases (Schmidt *et al*., 2004).

The main purpose of this study was to evaluate a) the antagonistic and potential biocontrol activities of the endophytic bacteria isolated from tomato roots against *Fusarium oxysporum* f.sp. *radicis-lycopersici* (FORL), and b) their growth promoting ability in tomato at *in vivo* conditions.

Materials and methods

Isolation of endophytic bacterial strains

Bacterial strains were isolated from tomato roots of different hybrid varieties from Algerian soil (Algiers locality). The roots were washed and dried aseptically. Root samples were surface-disinfected to remove epiphytes, using 95% ethanol for 30 seconds, followed by a 10% sodium hypochlorite treatment for 2 min and then 75% ethanol for

2 min. The root pieces were then rinsed three times with sterile distilled water to remove traces of disinfectant (Evans *et al*., 2003; Rubini *et al*., 2005). In a sterile mortar, the root pieces were crushed with sterile distilled water, to obtain a ground product including extracted bacterial cells. The enrichment was carried out by placing 1 g of the extract in 9 ml of nutrient broth. The culture was conducted at 30°C for 24 h, aiming to increase the initial biomass in order to recover a maximum bacterial charge (Bashan *et al*., 1993). 0.1 ml from each enrichment tube served to the isolation on nutrient agar at 30°C for 24 h. Once pure single colonies obtained, the conservation was achieved in glycerol at - 20°C.

Antagonistic activity of endophythic bacterial isolates against FORL

The test of the antifungal activity of the endophytic bacterial isolates against FORL was carried out on PDA medium using the dual culture technique described by Lee *et al*. (2010). A 6 mm fragment of FORL aged of 7 days was removed and deposited in the center of a new Petri dish containing the PDA medium and the bacterial suspensions were adjusted to 0.5 Mac Farland (prepared in nutrient broth). Then 5 µl of each tested bacterial suspension was placed at 1 cm from the edge of the same Petri dish. The experimental units were incubated at 25°C for 5 to 7 days. Control units included only the fungus without the tested bacteria. Each test was replicated three times. The inhibition rate was calculated as follows:

$$\textit{Inhibition rate (\%)} = \frac{X-Y}{X}100$$

Where:

X: Diameter of mycelium control (mm).
Y: Diameter of the mycelium in the presence of the bacterium (measured on the axis "fungus-bacterial colony" (mm).

Identification of endophytic bacterial isolates using 16S rRNA gene analysis

Total bacterial DNA was extracted according to the method of Liu *et al*. (2000). 16S rRNA gene fragments were amplified by PCR using an Invitrogen kit. Primers were as follows: 10-30 forward: 5'-GAG TTT GAT CCT GGC TCA-3' and 1500 reverse: 5'-AGA AAG GAG GTG CAG ATC CC-3'. 5 µl of 10 x PCR buffer (Mg^{2+}), 8 µl of deoxynucleotide triphos-phate (200 mM of each dNTP), 100 pM of each primer, 2 µl of template DNA solution and 0.8 µl of Taq enzyme (5 U/µl). DNA fragments were recovered and purified. Sequence determination was performed by Beckman Coulter Genomics (United Kingdom). After 16S rRNA gene sequencing, identification of phylogenetic neighbors was done using BLASTN program against databases containing type strains located at the EzBioCloud database (https://www.ezbiocloud.net/). Neighbor-joining method under MEGA6.0 package was used for the construction of phylogenetic trees. The 16S rRNA gene sequences were submitted to the NCBI GenBank database.

Screening endophytic bacterial isolates for growth promotion traits

Phosphate solubilization

Each bacterial isolate was inoculated in Pikovskaya agar containing: 10 g glucose, 5 g tribasic calcium phosphate ($Ca_5HO_{13}P_3$), 0.5 g $(NH_4)2SO_4$, 0.2 g KCl, 0.1 g $MgSO_47H_2O$, trace of $MnSO_4$ and $FeSO_4$, 0.5 g yeast extract, and 15 g agar, in 1000 ml distilled water (Pikovskaya, 1948.). After 7 days of incubation, the presence of clear halos around bacterial colony was used to indicate positive Phosphate (P) solubilizing strains (Husen, 2003).

Indole-3-acetic acid production (IAA)

Bacterial strains were inoculated into nutrient broth containing: 5 g peptone, 1.5 g yeast extract, 1.5 g beef extract, and 5 g NaCl, in 1000 ml distilled water supplemented with L-tryptophan (500 mg/L) and incubated at 30°C for 5 days. The supernatant of the stationary phase culture was obtained by centrifugation at 10000 rpm for 15 min (Bric *et al*., 1991). An aliquot of 2 ml supernatant was transferred to a fresh tube to which 5 ml of Salkowski's reagent (1 ml of 0.5 M Fe-

Cl_3 in 50 ml of 35% $HClO_4$) were added. After 25 min at room temperature, the absorbance of pink color developed was read at 530 nm. Varied amounts of pure indole-3-acitic acid were used as standard (Costacurta *et al*., 2006).

Siderophore production

All bacterial isolates were qualitatively screened for siderophore production by inoculation onto a Chrome Azurol Sulphonate (CAS) agar plate and incubation for 24 h at 28°C (Schwyn and Neilands, 1987). The change of the medium color from bluish to yellowish-orange after incubation indicates the presence of siderophores (Dias *et al*., 2017).

Impact of endophytic bacterial isolates on tomato plant growth

Seed treatments

Seeds of tomato (Isi Sementi S.p.A., Fidenza «Parma» Italy) were surface sterilized by soaking in 70% ethanol for 1 min followed by immersion in sodium hypochlorite (1%) solution for 10 min and rinsing at least five times with sterile distilled water. The seeds were germinated on sterilized filter paper sheets in the Petri dish (Piromyou *et al*., 2011). Each seed batch was inoculated with one of the selected isolates, using a suspension adjusted to 10^8 CFU/ml to evaluate their ability to modulate the plant response favorably (Piromyou *et al*., 2011).The bacterial treatment of the seeds was carried out under aseptic conditions by putting each batch of 22 seeds sterilized in the bacterial suspension for two different incubation periods (30 min and 60 min). The seeds of the "negative control" batches were inoculated with sterilized 0.85% NaCl solution and sown directly without bacterial treatment (Fallahzadeh-Mamaghani *et al*., 2009; Lee *et al*., 2010). The fungal suspension was made from fresh cultures of FORL, with a spore concentration of 10^6 conidia/ml (El Aoufir, 2001). The seeds were infested with FORL after the bacterial treatment, by immersing the batches in boxes filled with fungal suspension for a duration of 1 hour, then they have been recovered for sowing (Johansson *et al*., 2003).

Pot experiments

Pot experiments were conducted from March 2017 to April 2017. Tomato seeds which had undergone both bacterial and infestation treatment were sown in pots filled with a mixture of potting soil and sand (v/v) (approximately 100 g per pot) (Lee *et al*., 2010), Twenty two seeds per strain were used in four replicates. Pot plants were sprayed with distilled water using a spray gun and incubated at room temperature (20 to 25°C) and photoperiod 9:15 hours L:D. After 31 days of culture, the evaluation of the impact of fungal and bacterial treatment on the plant growth was made by measuring the length of the stem and main root, and the dry weight of each treated batch.

Statistical analysis

ANOVA with Duncan's multiple range test was used to detect significant differences of the experimental data using XLSTAT software, version 2014.3.01 (Adinosoft).

Results and discussion

Selection of antagonistic bacteria

The Dual Plate Assay revealed the presence of isolates capable of inhibition or restriction of the growth of FORL. The inhibition rates obtained were: EB4B (51.51%), EB22K (51.18%) and EB2A (41.40%) (Fig. 1), indicating that the strains possess significant antagonistic effect towards the pathogen. A relatively broader inhibition zone involves the synthesis of relatively potent antibiotics (Kadir, 2008). Lee *et al*. (2010) were able to isolate bacterial strains which were considered to have good antifungal potential with inhibition rates ranging from 0 to 45%. Mikani *et al*. (2007) results in the *Pseudomonas fluorescens* antagonism tests showed an inhibition of mycelial growth with an average of 59.8% for the best performing strain. Bacteria can produce many antifungal metabolites (Weller *et al*., 2007) such as phenazines, pyoluteorin, pyrrolnitrin, DAPG (2,4-diacetyl

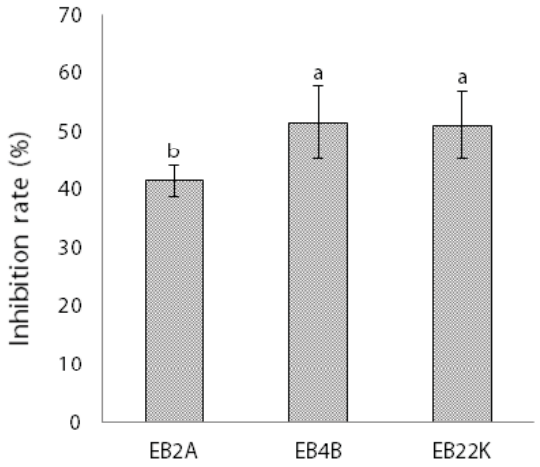

Figure 1. Dual Plate Assay against *Fusarium oxysporum* f.sp. *Radicis lycopersici*. Inhibition rate (%) caused by *Leclercia adecarboxylata* (EB2A), *Enterobacter ludwigii* (EB4B) and *Enterobacter ludwigii (*EB22K) isolates. Data are presented as means ±SE. ANOVA with Duncan's multiple range test was used to detect significant differences. Bars with different letters within the parameter indicate significant differences at $P \leq 0.05$.

phloroglucinol) and siderophores (pyoverdines or pseudobactins) which are the most frequently detected antifungals (Haas and Défago, 2005; Lemanceau *et al*., 2009).

Identification of selected PGPRs

The 16S rRNA gene sequences of the three bacterial strains reported in this paper are deposited in NCBI with the GenBank, with the following accession numbers: EB2A (MF693122), EB4B (MF693530) and EB22K (MF706259). Based on 16S rRNA gene sequence analysis, the isolates EB4B and EB22K were closely related to *Enterobacter ludwigii* EN-119 with an homology of 99.28% and 98.69%, respectively (Fig. 2).According to 16S rRNA sequence, the strain EB2A showed close proximity with *Leclercia adecarboxylata* NBRC 102595 (99.71%) (Fig. 2).

Non plant pathogenic endophytic bacteria can promote plant growth, improve nitrogen nutrition, and in some cases, are human pathogens such as enteric bacteria (Tyler and Triplett, 2008). PGPRs in different field crops are considered as human opportunistic pathogens (Dutta and Thakur, 2017). The third group of microorganisms that can be found in the rhizosphere are true and opportunistic human pathogenic bacteria, which can be carried on or in plant tissue and may enhance plant growth and health, in particular the *Enterobacteriaceae* that can invade the root tissue (Mendes *et al*., 2013). All strains isolated in this study should be further analysed using multi locus analysis to further species clarification. Thus, we can have a safer view of whether they are actually potential human pathogens or not.

Growth promotion traits of selected PGPRs

Bacterial isolates EB2A, EB4B and EB22K showed a distinct zone with the appearance of orange color indicating the production of siderophore which is beneficial to plants, via potential increase of iron availability. All isolated strains were able to solubilize the tricalcium P, resulting in large clear/halo zones.

Indolic acetic acid production was not remarkably different in the three bacterial strains (EB2A = 140 ± 0.10 µg/mL, EB4B = 152 ± 0.44 µg/mL and EB22K = 155 ± 0.78 µg/mL). Various plant growth promoting *Enterobacter* spp. such as *Enterobacter ludwigii* have been applied for plant development (Shoebitz *et al*., 2009; Madhaiyan *et al*., 2010; Kapoor *et al*., 2017). Shoebitz *et al*. (2009) and Gopalakrishnan *et al*. (2012) have also reported the exhibition of nitrogenase activity, phosphate solubilisation, IAA production and antifungal activity by a *E. ludwigii* strain. Previous reports had described some *Leclercia adecarboxylata* as efficient PGPR (Naveed *et al*., 2014; Melo *et al*., 2016; Kisiel and Kepczynska, 2016). Naveed *et al*. (2014) reported that inoculation with *Leclercia adecarboxylata* showed statistically significant greater biomass than the controls under environmental chamber conditions.

Siderophores production has been shown (CAS positive reaction), highlighting the potential of the strains belonging to *Enterobacter* genus to produce such secondary metabolites. Plants are known to use various bacterial siderophores as an iron source (Martinez-Viveros *et al*., 2010).

All three strains were capable of solubilizing the insoluble tricalcium phos-

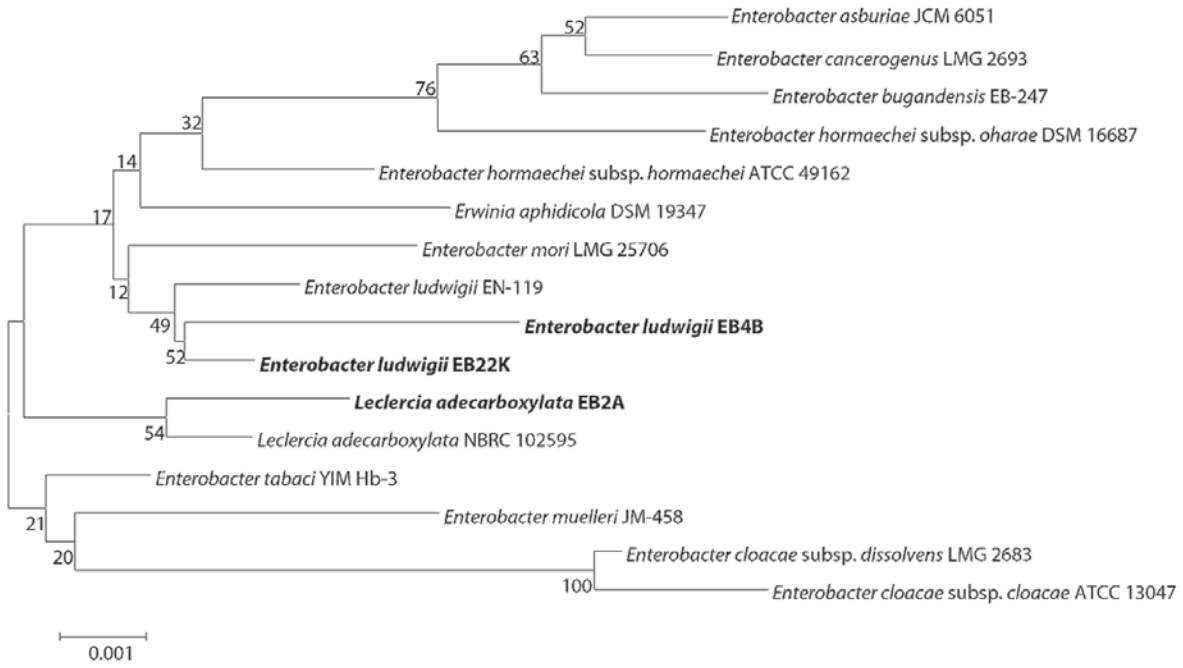

Figure 2. Phylogenetic analysis of *Leclercia adecarboxylata* (EB2A), *Enterobacter ludwigii* (EB4B) and *Enterobacter ludwigii* (EB22K) based on 16s rRNA gene sequencing. Neighbor joining tree was created using MEGA 6 (1000 bootstrap replicates) with a scale of 0.001 substitutions per nucleotide position.

phate ($Ca_5HO_{13}P_3$). Phosphate (P)-solubilizing bacter ia can exert a positive effect on tomato growth, increasing the phosphorus status (Van Veen *et al*., 1997) and significantly enhance photochemical activity accompanied by an increase of chlorophyll content in plants (Liu *et al*., 2017).

Production of siderophores (Loaces *et al*., 2011), solubilization of mineral phosphates (Ahemad *et al*., 2008), and synthesis of indolic acetic acid (IAA) (Khan *et al*., 2014) have beneficial effects on plant growth and are considered as frequent characteristics of PGPR (Gupta *et al*., 2012).

Effect of PGPRs on root size and plant biomass

The analysis of root length and dry weight after 31 days of culture showed that a period of 30 min of bacterization led to significantly better results than those with 60 min and revealed that inoculation of the PGPRs resulted in a significant increase in the biomass compared to the uninoculated controls. Inoculation with the strain EB4B significantly increased the root length compared to non-inoculated tomato seeds. The root length measurements showed a major increase of 326.60% for EB4B and 144.33% for EB22K (Fig. 3a) compared to control batches. The non-treated plants resulted in lower dry weight (Fig. 4a), while the treated exhibited an increase of 142.70% for EB4B and 79.94% for EB22K. After 31 days of cultivation, the plants of the "Control +" batch (Seeds in presence of the pathogen) experienced a low development compared to the "Control -" batch, which demonstrates the virulence of FORL and its incidence on tomato plants growth.

Treatment of tomato seeds with the endophytic strains in the presence of FORL resulted in significantly higher length of roots and plant dry weight compared to control, implying an increase of 180.81% for EB4B batch (Fig. 3b) whilst the average dry weight was enhanced by 202.15% for the same treatment (Fig. 4b). Thus, a significantly strong protective potential against FORL is indicated.

Overall, the strains exerted a particularly positive effect on the root system de-

velopment by increasing *in vivo* root elongation and plant biomass (dry weight). The effect depended on the exposure time of the seeds to the inocula. A longer exposure (after 60 min of contact) produced a negative effect on root elongation and plant development, while a low bacterial concentration (after 30 min of contact) increased the root elongation and plant biomass. Kisiel and Kepczynska (2016) also reported that a higher density of the inoculum produced a negative effect on root elongation.

The increase in crop yield due to a bacterial culture results from two main beneficial effects: the stimulation of plant growth and the protection of plants against soilborne diseases. PGPR treatments are known to increase the percentage of germination, seedling vigor, emergence, root and stem development, total plant biomass, seed weight, early flowering, and yields of fruits and seeds (Ramamoorthy *et al*., 2001). The colonization of the roots by endophytic bacteria introduced on the seeds is distributed along the roots, including partial or complete colonization of the rhizosphere: inside the root, on the root surface and in the immediate soil of the rhizosphere (Landa *et al*., 2001). Kokalis-Burelle (2002) and Vessey (2003) reported that PGPR could increase yield of tomato by increasing the availability of nutrients in rhizosphere soil and promoting other beneficial plant-growth promoting bacteria. PGPRs can remarkably increase nutrient availability in inoculated plants in plots compared to non-inoculated ones (Adesemoye *et al*., 2008) and lead to a better tomato growth. Karlidag *et al*. (2013) demonstrated that inoculation of selected PGPR increased considerably the growth, chloro-

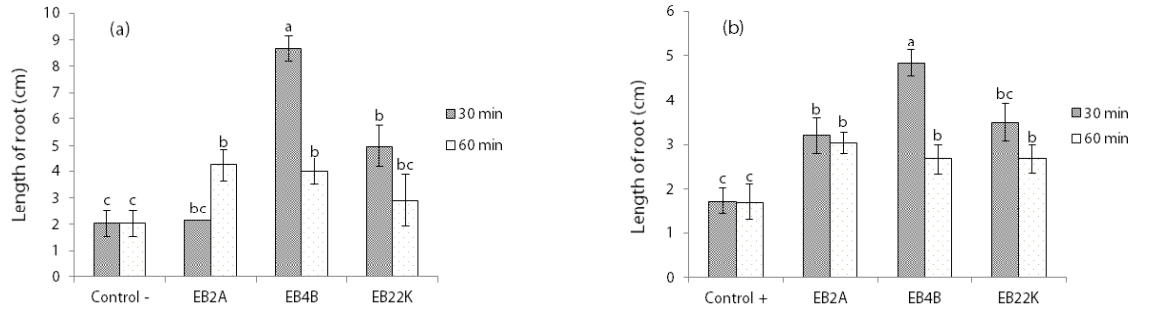

Figure 3. Growth promotion of tomato root induced by *Leclercia adecarboxylata* (EB2A), *Enterobacter ludwigii* (EB4B) and *Enterobacter ludwigii* (EB22K) isolates (30 min and 60 min of contact) a) in the absence of FORL and b) in the presence of FORL evaluated after 31 days. Data are presented as means ±SE. ANOVA with Duncan's multiple range test was used to detect significant differences. Bars with different letters within the parameter indicate significant differences at $P \leq 0.05$.

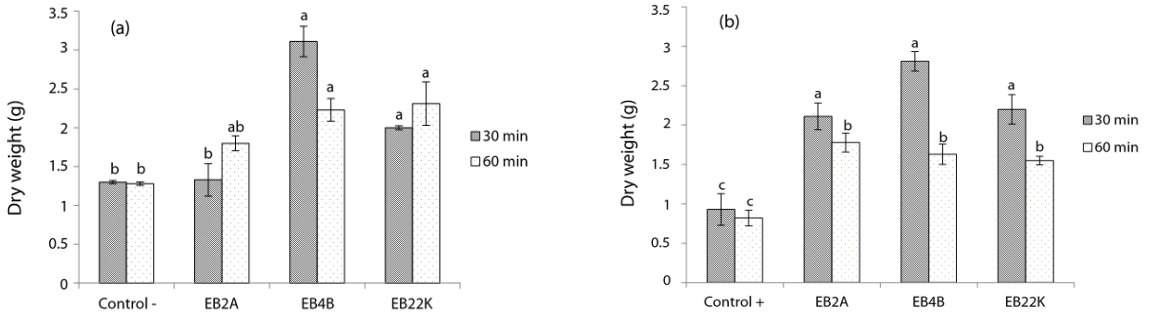

Figure 4. Dry weight promotion of tomato plants induced by *Leclercia adecarboxylata* (EB2A), *Enterobacter ludwigii* (EB4B) and *Enterobacter ludwigii* (EB22K) isolates (30 min and 60 min of contact) a) in the absence of FORL and b) in the presence of FORL evaluated after 31 days. Data are presented as means ±SE. ANOVA with Duncan's multiple range test was used to detect significant differences. Bars with different letters within the parameter indicate significant differences at $P \leq 0.05$.

phyll content and nutrient.

The treatment of tomato seeds with PGPR strains (*Leclercia adecarboxylata* and *Enterobacter ludwigii*) have led to a reduction in susceptibility to FORL, with a substantial promotion of tomato growth (length of root and dry weight). The protective effect of the strains could be explained by their ability to produce antifungal substances, as highlighted by previous antifungal potential characterization studies (bacteria producing salicylic acid, rhamnolipids, chitinase, cellulases) in addition to good competitiveness for carbon and energy sources (Haas and Défago, 2005).

In vivo tests indicate the protective effect of the isolated PGPR against FORL in the tested tomato cultivar, but also growth promotion with singular rates up to 200%. For such reason and for other reasons, root colonization is often considered as a limiting factor for biological control in the rhizosphere (Dekkers *et al*., 1997). However, selection of root colonizers has been an empirical process involving the random control of isolates. Herein, the relationship between growth promotion and protection of tomato will allow more targeted selection of strains for more effective use in biological control (Landa *et al*., 2001). The use of *Leclercia adecarboxylata* and *Enterobacter ludwigii* has already been reported for growth promotion and protection of tomato (Gopalakrishnan *et al*., 2012; Kisiel and Kepczynska, 2016), and the traits and genes that contribute to root colonization capacity have been extensively studied (Landa *et al*., 2001). Nevetheless, the relationship between protection and the growth promotion or yield enhancement by PGPR has been further established, as it appears that the reduction in the severity of the disease is accompanied by an increase in the yield after adequate bacterial treatments (Lemanceau and Alabouvette, 1991).

In conclusion, biological control of root and crown rot disease caused by the FORL soil pathogen in tomato, *via* the introduction of the tested endophytic bacterial strains of *Enterobacter* species, is proposed as a potential alternative to chemical substances. Based on antagonistic tests, the bacterial isolates with remarkable beneficial traits such as the combination of production of IAA, siderophores and phosphate solubilization could be a useful tool to enhance the sustainable production of tomato. *In vivo* trials demonstrated an efficient interaction of the three isolates with the tomato plant, promoting growth and induction of plant defense against FORL. Isolate EB4B appears to be a promising alternative for future bioformulation and field application.

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Επίδραση εφαρμογής του ενδοφυτικού βακτηρίου *Enterobacter ludwigii* EB4B στην προώθηση της ανάπτυξης φυτών τομάτας

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Περίληψη Η μελέτη αυτή στοχεύει στην ανάπτυξη ενός βιολογικού παράγοντα έναντι του *Fusarium oxysporum* f.sp. *radicis-lycopersici* (FORL) στην τομάτα, ο οποίος παράλληλα προωθεί την ανάπτυξη των φυτών. Για το σκοπό αυτό, εξετάστηκε ένα σύνολο 23 ενδοφυτικών βακτηριακών στελεχών για την ικανότητά τους να αναστέλλουν την ανάπτυξη του FORL χρησιμοποιώντας τη δοκιμασία διπλής καλλιέργειας σε τρυβλίο. Τρία απομονωθέντα στελέχη με την πιο ανταγωνιστική δράση προς το FORL εξετάστηκαν επίσης ως προς την παραγωγή σιδηροφόρων, τη διαλυτοποίηση φωσφορικών αλάτων και τη σύνθεση ινδολοξικού οξέος (IAA) ως χαρακτηριστικά της προώθησης της ανάπτυξης. Η ικανότητα αναστολής της ανάπτυξης του FORL ήταν αντίστοιχα 51,51% (EB4B), 51,18% (EB22K) και 41,40% (EB2A). Με βάση την ανάλυση αλληλουχίας του γονιδίου 16S rRNA, τα στελέχη EB4B και EB22K κατατάσσονται φυλογενετικά πλησίον του στελέχους *Enterobacter ludwigii* EN-119, ενώ το στέλεχος EB2A έχει αποδοθεί στο *Leclercia adecarboxylata* NBRC 102595. Η επίδραση των στελεχών EB2A, EB4B και EB22K στην προώθηση ανάπτυξης των φυτών της τομάτας εξετάστηκε *in vitro* παρουσία του φυτοπαθογόνου FORL. Οι επεμβάσεις με τα επιλεγμένα στελέχη αύξησαν σημαντικά το μήκος της ρίζας και το ξηρό βάρος των

φυτών. Τα πιο ενθαρρυντικά αποτελέσματα έδωσε το στέλεχος EB4B απουσία του FORL αυξάνοντας το μήκος της ρίζας κατά 326,60% και το ξηρό βάρος κατά 142,70% σε σύγκριση με τους μάρτυρες. Παρουσία του FORL, το στέλεχος EB4B βελτίωσε τόσο το μήκος της ρίζας (180,81%) όσο και το ξηρό βάρος (202,15%) των φυτών της τομάτας. Τα αποτελέσματα ενισχύουν την περαιτέρω μελέτη της παρατηρούμενης ευεργετικής επίδρασης του *Enterobacter* sp. EB4B για πιθανή χρήση του ως βιοδιεργερτικό παράγοντα και παράλληλα παράγοντα βιολογικής καταπολέμησης του FORL.

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