Antagonist activity of bacteria isolated from "*Solanum elaeagnifolium*" green compost against *Fusarium oxysporum* f.sp.*radicis-lycopersici*

Atividade antagonista de bactérias isoladas do composto verde "*Solanum elaeagnifolium*" contra *Fusarium oxysporum* f.sp.*radicis-lycopersici*

Actividad antagonista de bacterias aisladas del compost verde «*Solanum elaeagnifolium*» frente a *Fusarium oxysporum* f.sp.*radicis-lycopersici*

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ABSTRACT

In the present study, 14 bacteria were isolated from "*Solanum elaeagnifolium"* green compost belonging to 12 species: *Paenibacillus macerans, Bacillus circulans, Geobacillus thermoglucosidasius, Bacillus licheniformis, Serratia liquefaciens, Bacillus megaterium, Paenibacillus glucanolyticus, Serratia marescens, Pseudomonas aeruginosa, Sporosarcina pasteurii, Lysinibacillus sphaericus* and *Brevibacillus laterosporus.* For *Pseudomonas aeruginosa* and *Brevibacillus laterosporus,* two different strains were obtained for each species. All the 14 isolates were tested for their antagonistic activity on *Fusarium oxysporum* f.sp.*radicis-lycopersici* the causal agent of tomato crown and root rot (FCRR). The assessment of the *in vitro* interactions between bacterial strains and the phytopathogenic fungi was performed by dual confrontation assays on PDA medium. All the 14 bacteria were antagonistic against the phytopathogen, with an inhibitory effect varying between 67,14% and 52,85%. *Sporosarcina pasteurii* and *Brevibacillus laterosporus* (S12-2) showed antifungal effect higher than the other bacteria, where they exhibit an significant reduction in fungal mycelium growth about 67,14%. The presences of different antagonist species of bacteria witnessed the maturity of the green compost and make it as a good organic amendment for tomato plant culture for stimulate their growth and to protect them from FORL. The inventory of the bacterial community of this compost was necessary to determine its quality for an appropriate application of them in future as a biological control agent against FORL in tomato cultivation.

Keywords: *Solanum elaeagnifolium,* green compost, antagonistic bacteria, antifungal activity, mycelial growth, *Fusarium oxysporum* f.sp *radicis-lycopersici*

RESUMO

No presente estudo, 14 bactérias foram isoladas do composto verde "*Solanum elaeagnifolium*", pertencentes a 12 espécies: *Paenibacillus macerans, Bacillus circulans, Geobacillus thermoglucosidasius, Bacillus licheniformis, Serratia liquefaciens, Bacillus megaterium, Paenibacillus glucanolyticus, Serratia marescens, Pseudomonas aeruginosa, Sporosarcina pasteurii, Lysinibacillus sphaericus* e *Brevibacillus laterosporus*. Para *Pseudomonas aeruginosa* e *Brevibacillus laterosporus*, foram obtidas duas cepas diferentes para cada espécie. Todos os 14 isolados foram testados quanto à sua atividade antagônica em *Fusarium oxysporum* f.sp.*radicis-lycopersici*, o agente causal da podridão da coroa e da raiz do tomateiro (FCRR). A avaliação das interações in vitro entre as cepas bacterianas e os fungos fitopatogênicos foi realizada por meio de ensaios de confronto duplo em meio PDA. Todas as 14 bactérias foram antagônicas contra o fitopatógeno, com um efeito inibitório variando entre 67,14% e 52,85%. A *Sporosarcina pasteurii* e a *Brevibacillus laterosporus* (S12-2) apresentaram efeito antifúngico superior ao das outras bactérias, exibindo uma redução significativa no crescimento do micélio fúngico de cerca de 67,14%. As presenças de diferentes espécies antagonistas de bactérias testemunharam a maturidade do composto verde e o tornaram um bom aditivo orgânico para a cultura do tomateiro, estimulando seu crescimento e protegendo-o contra a FORL. O inventário da comunidade bacteriana desse composto foi necessário para determinar sua qualidade para uma aplicação adequada no futuro como agente de controle biológico contra a LAR no cultivo de tomate.

Palavras-chave: *Solanum elaeagnifolium,* composto verde, bactérias antagônicas, atividade antifúngica, crescimento micelial, *Fusarium oxysporum* f.sp *radicis-lycopersici*

RESUMEN

En el presente estudio, se aislaron 14 bacterias del compost verde «*Solanum elaeagnifolium*» pertenecientes a 12 especies: *Paenibacillus macerans, Bacillus circulans, Geobacillus thermoglucosidasius, Bacillus licheniformis, Serratia liquefaciens, Bacillus megaterium, Paenibacillus glucanolyticus, Serratia marescens, Pseudomonas aeruginosa, Sporosarcina pasteurii, Lysinibacillus sphaericus* y *Brevibacillus laterosporus*. En el caso de *Pseudomonas aeruginosa* y *Brevibacillus laterosporus*, se obtuvieron dos cepas diferentes para cada especie. Se comprobó la actividad antagonista de los 14 aislados sobre *Fusarium oxysporum* f.sp.*radicis-lycopersici*, el agente causal de la podredumbre de la corona y la raíz del tomate (FCRR). La evaluación de las interacciones in vitro entre las cepas bacterianas y los hongos fitopatógenos se realizó mediante ensayos de doble confrontación en medio PDA. Las 14 bacterias resultaron antagonistas frente al fitopatógeno, con un efecto inhibitorio que osciló entre el 67,14% y el 52,85%. *Sporosarcina pasteurii* y *Brevibacillus laterosporus* (S12-2) mostraron un efecto antifúngico superior al de las otras bacterias, donde exhibieron una reducción significativa del crecimiento del micelio fúngico en torno al 67,14%. La presencia de diferentes especies de bacterias antagonistas atestiguan la madurez del compost verde y lo convierten en una buena enmienda orgánica para el cultivo de tomateras para estimular su crecimiento y protegerlas del FORL. El inventario de la comunidad bacteriana de este compost era necesario para determinar su calidad para una adecuada aplicación de las mismas en el futuro como agente de control biológico contra el FORL en el cultivo de tomate.

Palabras clave: *Solanum elaeagnifolium,* compost verde, bacterias antagonistas, actividad antifúngica, crecimiento micelial, *Fusarium oxysporum* f.sp *radicis-lycopersici*

1 INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important economical crops in Algeria as in many countries over the world. *Fusarium* crown and root rot in tomato plants caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* is the most fungi disease causing significant yield losses in tomato cultivation (Saidi et al., 2009; Innocenti et al., 2015). Biological control of plant pathogens is an attractive alternative to chemical fungicides (Ozbay and Newman, 2004; Hibar et al., 2006; Avis, 2007), which cause environmental pollution, soil deterioration, devastation of the beneficial rhizospheric microorganisms, development of resistant strains and having a high risk on humans, animals and plants health (Chambers et al., 2014; Hahn, 2014). The use of composted agricultural wastes has been reported by several previous studies to suppress soil-borne pathogens and improve plant growth (Pharand et al., 2002; Hibar et al., 2006; Shanying et al., 2016; Ros et al., 2020; Merah et al., 2022). The beneficial effect of compost is related to its microbial community (fungi and bacteria). The antagonistic bacteria isolated from compost have been widely studied. The most studies have focused on their antagonist mechanisms such as competition for nutrients or iron, competition for infection sites on roots, or production of antibiotics (Weller, 2007; Whipps et al., 2008). Species of *Pseudomonas*, *Bacillus* and *Serratia* are the most known for their ability to reduce plant diseases caused by soil-borne fungal pathogens (Iwamoto and Aino, 2008; Johnson and Dunlap, 2019; Clements et al., 2019). Fluorescent *Pseudomonas* species are recognized as biocontrol agents basing on their excellent root colonizing ability, production of a wide range of antifungal metabolites and their catabolic adaptability (Iwamoto and Aino, 2008). A number of *Bacillus* species and those from the order *Bacillales* have been recognized by their antagonist activity against tomato phytopathogens (Pane and Zaccardelli, 2015; Solanki et al., 2019; Guevara-Avendano et al., 2018; Burkett-Cadena et al., 2019; Johnson and Dunlap, 2019).The antagonistic activity of *Bacillus* species is related to their ability to produce extracellular chitinolytic enzymes, antibiotic compounds and to efficiently compete for space and nutrients in the rhizosphere (Zhao et al., 2013). Members of the genus *Serratia* are also of great research interest because they are almost ubiquitous with antimicrobial properties (Su et al., 2016; Clements et al., 2019). They are able to produce freely diffusible compounds with fungi-static activity *in vitro* confirmed their biocontrol efficacy on fungi pathogens (Michail et al., 2022).

The principal objective of the present study is to isolate, identify and evaluate *in vitro* the antagonist effect of bacterial strains obtained from "*Solanum elaeagnifolium"* green compost against FORL, which could then be considered useful as biocontrol agents.

2 MATERIALS AND METHODS

2.1. *SOLANUM ELAEAGNIFOLIUM* GREEN COMPOST PREPARATION

Green compost was prepared with 100% of debris plants of "*Solanum elaeagnifolium"* (an invasive plant) according to an aerobic-mesophilic process using "Surface composting" method during the period 2016-2017 at USTO-MB University. Surface composting was carried out with periodical turning (Indore method) over a period of 06 months. It pH (extracted in the water) is 7.4, the ration C/N is about 14.5 and its salinity is about 3,75 (mS/cm).

2.2. PREPARATION OF COMPOST DILUTIONS

To evaluate bacteria quantification in the green compost, the technic of soil dilution plating was adapted from method by Rapilly (1968). 10g of dried compost was homogined in 90ml of sterilised distilled water and shaken well for 30 min. The dilution factor for this solution is 10^{-1}. Next, 1ml of the suspension was removed from the previous solution using a sterile pipette and then transferred to a new tube containing 9ml of sterilised distilled water. The tube was capped and vortexed. The dilution factor for this solution is 10^{-2}. The dilution series was continued to obtain solutions with dilution factors of 10^{-3}, 10^{-4} and 10^{-5}.

2.3 CULTURE MEDIUM

The medium used to cultivate the different bacteria isolated from the green compost was Nutrient Agar (Beef extract: 1g; yeast extract: 2,5g; peptone: 5g; Sodium Chloride: 5g; 15g of agar-agar; distilled water 1000 ml).The medium was autoclaved at 121°C for 15 minutes. After autoclaving, an antibiotic (10 mg of Cycloheximide) was added to 100 ml of cooled molten Nutrient Agar media to avoid fungal development.

2.4 BACTERIA DENOMBREMENT

For bacteria denombrement, 1 ml of each compost dilution was transferred into Petri dish containing molten Nutrient Agar supplemented with 10mg/100ml of Cycloheximide. The medium and inoculums were homogenized before the agar solidifies. After all plates have solidified, they were incubated at 28°C for 48 hours. Each individual dilution was replicated thrice. The whole experiment was repeated twice.

2.5 RESULTS EXPRESSION

The detremination of green compost bacteria charge was done by the comptage of bacteria colonies and results were given using the expression of "CFU" (Colony Forming Unit) /g of green compost, and were calculated according to the following formula:

$$N = \frac{\sum \text{colonies}}{\text{Vml x } (\text{n1} + 0{,}1\text{n2})\text{x d1}}$$

Where N: Numer of CFU per gramme of green compost; ∑ colonies: all colonies in taken Petri dishes; V: Volume of disposed solution (1ml); n_1: number of petri dishes taken in the first dilution; n_2: number of petri dishes taken in the second dilution; d_1: factor of the first dilution taken.

Only Petri dishes having between 15 and 150 colonies in two successive dilutions were taken for denombrement (Dutruc-Rosset, 2003). The number of Colony Forming Unit per g of dry green compost was calculated for the total bacteria load and for each species.

2.6 ISOLATION, IDENTIFICATION AND SCREENING OF BACTERIA FOR ANTAGONISTIC ACTIVITY

After 48 hours of incubation at 28°C, the most numerous bacterial colonies with visually different morphology were picked out and isolated. To obtain the pure culture, these isolates were transferred to other dishes on Nutrient Agar media supplemented with Cycloheximide, then, incubated at 28°C for 48 hours. Bacteria strains are tested on Kings B (KB) agar and identified using macroscopic, microscopic and biochemical characters using Api 20E gallery. Species were identified on line by UPBM le lab. 2017-2024 Software. All bacteria isolates were screened for antagonistic activity against FORL.

2.6.1 Fungal pathogen

In this study, FORL strain used was isolated in 2013, from tomato plants cultivated under greenhouse from north-west Algeria and showing a high level of pathogenicity based on typical symptoms of *Fusarium* Crown and Root Rot (FCRR) disease. After its macroscopic and microscopic identification, the determination of *forma specialis* of this pathogen was done using pathogenicity test on two different tomato cultivars (Rio Grande and MontFavet) basing on his sensibility against FORL. Fungal cultures were grown on Potato Dextrose Agar (PDA) medium and incubated at 28°C for 7 days before being used for antifungal assays.

2.6.2 Antagonist effect of bacterial isolates

For the evaluation of antagonist effect of bacterial isolates against FORL, a dual culture technique on PDA (Potato Dextrose Agar) medium was used. A mycelium disk of 5 mm diameter was cut out from periphery of actively growing fungus FORL (4-7 days old culture) on agar plate and placed on the surface of fresh agar medium at 3 cm radius, diametrally opposed the isolated bacteria (24 h old) by streaking in a straight line on one edge of medium in Petri plate (90 mm diameter). Plates inoculated without bacteria were used as control.

Petri dishes were then incubated at 28°C for 5 days. After that, radial growth of fungus was measured and Per cent inhibition of fungi (growth reduction over control) was calculated by the following equation:

$$I\ (\%) = D1 - D2 / D1 \times 100$$

I (%) = inhibition of mycelial growth; D1= radial growth of fungus in control plate (mm); D2= radial growth of fungus on the plate inoculated with bacteria (mm).

For each treatment three replicates were maintained and repeated twice.

2.7 STATISTICAL ANALYSIS

An analysis of variance (ANOVA), using SPSS (Statistical Package for the Social Sciences) version 16.0., associated to the mean comparison test of Student-Newman-Keuls (SNK) (at $P \leq 0.05$) was performed to attest the *in vitro* growth rates of the fungal strains.

3 RESULTS AND DISCUSSION

3.1 BACTERIA QUANTIFICATION

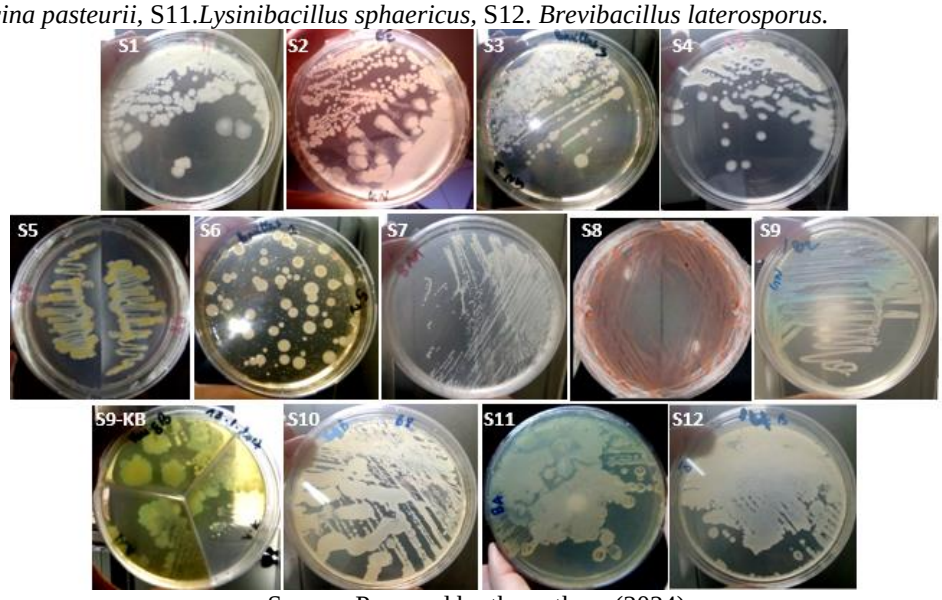
Total bacterial load in green compost was high. It was about 8,71 $x10^6$ CFU/g of dried green compost. 12 different macroscopic aspects of colonies were observed and 14 strains were obtained.

3.2 ISOLATION AND IDENTIFICATION OF BACTERIA STRAINS

To identify the 14 bacteria strains isolated from "*Solanum elaeagnifolium"* green compost, bacterial colonies obtained were purified after selection based on macroscopic aspect. The observations of these colonies showed 12 kinds of colonies (Fig.1). Then, a phenotypic identification using Api 20E gallery was used. Species identification was done on line by UBPM le lab.2017-2024 software (Fig.2). 12 bacterial species were identified belonging to eight genuses: *Paenibacillus, Bacillus, Geobacillus, Serratia, Pseudomonas, Sporosarcina, Lysinibacillus* and

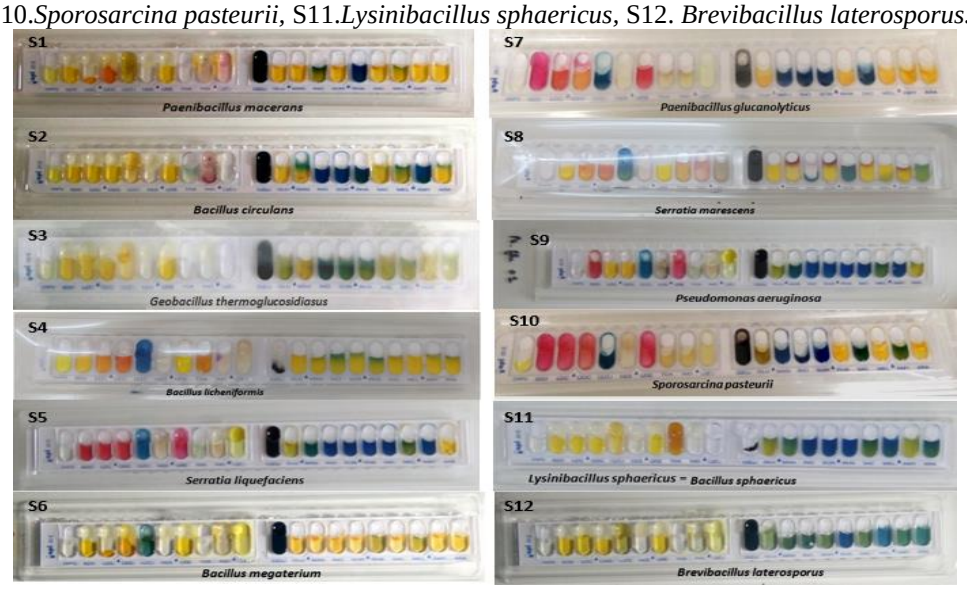
Brevibacillus. Each species charge in green compost is showed on table1. The highest concentration is represented by *Bacillus licheniformis* $1{,}0x10^4$ CFU/g of dried compost, while the less concentration is noted with *Pseudomonas aeruginosa* 1,4 and $1{,}7x10^2$ CFU/g dc. In fact, the isolation of an important number of bacterial species witnessed the diversity of bacteria population of the studied green compost.

Figure 1: Macroscopic aspect of the 12 representative colonies of the 14 bacterial strains isolated from "*Solanum elaeagnifolium"* green compost. On nutrient agar: S1. *Paenibacillus macerans,* S2. *Bacillus circulans,* S3.*Geobacillus thermoglucosidasius,* S4.*Bacillus licheniformis,* S5.*Serratia liquefaciens,* S6.*Bacillus megaterium,* S7.*Paenibacillus glucanolyticus,* S8.*Serratia marescens,* S9.*Pseudomonas aeruginosa,* S9-KB.*Ps.aeruginosa* on king B, S10.*Sporosarcina pasteurii,* S11.*Lysinibacillus sphaericus,* S12. *Brevibacillus laterosporus.*



Source: Prepared by the authors (2024)

Figure 2: Phenotypic identification of the 12 bacterial strains isolated from "*Solanum elaeagnifolium"* green compost, using Api 20^E gallery. Species were identified on line by UPBM le lab. 2017-2024 Software. S1. *Paenibacillus macerans,* S2. *Bacillus circulans,* S3.*Geobacillus thermoglucosidasius,* S4.*Bacillus licheniformis,* S5.*Serratia liquefaciens,* S6.*Bacillus megaterium,* S7.*Paenibacillus glucanolyticus,* S8.*Serratia marescens,* S9.*Pseudomonas aeruginosa,* S10.*Sporosarcina pasteurii,* S11.*Lysinibacillus sphaericus,* S12. *Brevibacillus laterosporus.*



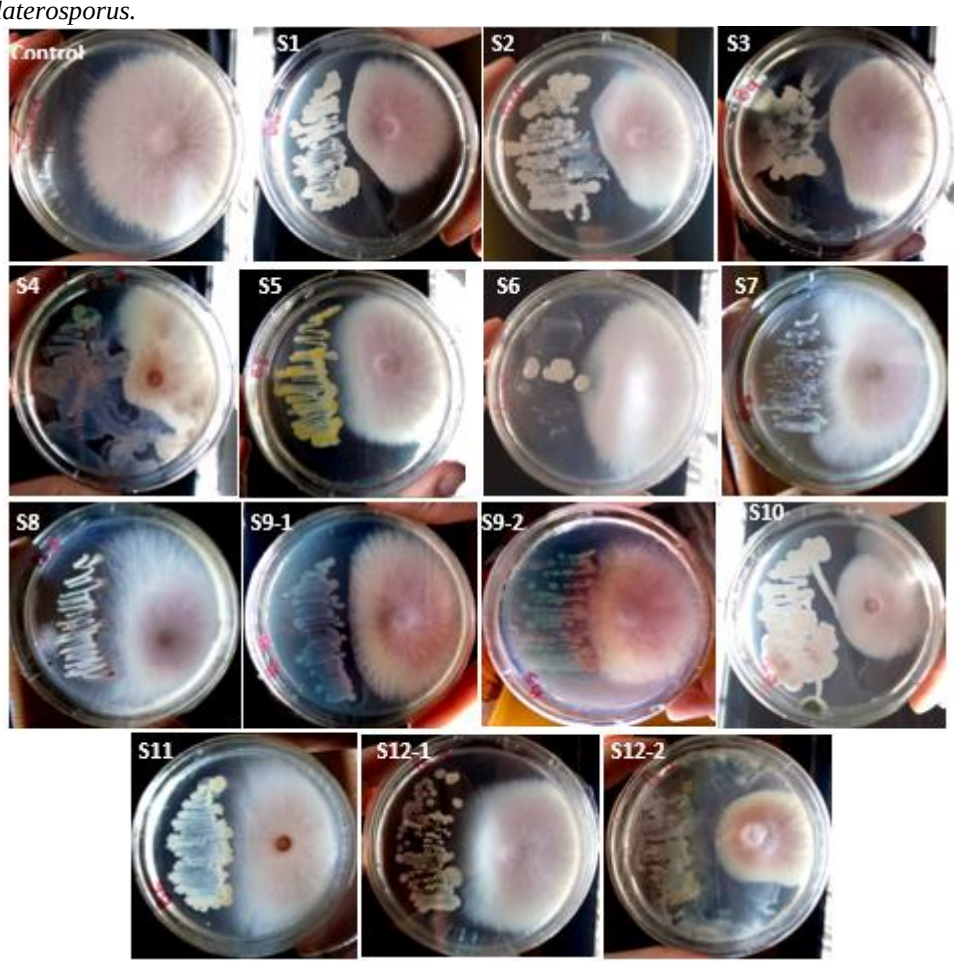
Source: Prepared by the authors (2024)

3.3 ANTAGONIST EFFECT OF BACTERIAL ISOLATES

From"*Solanum elaeagnifolium"* green compost, 14 bacterial isolates belonging to 12 species were obtained, with two strains of *Pseudomonas aeruginosa* and two strains of *Brevibacillus laterosporus* (table1). All the 14 bacterial isolates were tested for their antagonist activity using direct confrontation with FORL on PDA medium.

The direct confrontation of the 14 bacterial isolates with FORL, showed an inhibitory effect on mycelium growth of the fungal pathogen (Fig.3).

Figure 3: Antagonist activity of bacterial strains isolated from "*Solanum elaeagnifolium"* green compost on FORL mycelium growth on PDA medium after 5 days of incubation at 28°C. S1. *Paenibacillus macerans*, S2.*Bacillus circulans*, S3.*Geobacillus thermoglucosidasius*, S4.*Bacillus licheniformis*, S5.*Serratia liquefaciens*, S6.*Bacillus megaterium*, S7.*Paenibacillus glucanolyticus*,S8.*Serratia marescens*, S9-1.*Pseudomonas aeruginosa*, S9-2.*Ps.aeruginosa*, S10. *Sporosarcina pasteurii*, S11.*Lysinibacillus sphaericus* ,S12-1.*Brevibacillus laterosporus*, S12-2. *Brevibacillus laterosporus.*



Source: Prepared by the authors (2024)

Results showed that all the 14 isolates have an inhibitory effect on FORL mycelium growth (Fig.3). The highest inhibition 67,14% was observed with *Sporosarcina pasteurii* and *Brevibacillus laterosporus* (S12-2) . An inhibitory effect about 64,28%, 61,42% and 60% were noted with *Bacillus licheniformis, Paenibacillus macerans* and *Bacillus circulans* respectively.

Geobacillus thermoglucosidasius, *Serratia liquefaciens* and *Brevibacillus laterosporus* (S12-1) exhibit an inhibitory effect about 58,57%, where 57,14% were observed with *Bacillus megaterium* and *Pseudomonas aeruginosa* (S9-1). The less effect 52,85% was noted with *Paenibacillus glucanolyticus*, *Serratia marescens* and *Lysinibacillus sphaericus*. The antagonistic isolates tested in this study were grouped into eight genera (*Paenibacillus, Bacillus, Geobacillus, Serratia, Pseudomonas, Sporosarcina, Lysinibacillus* and *Brevibacillus*) (table1).

Table1. Identification, antagonist effects on FORL mycelium growth and load of bacterial isolates.

Bacterial isolates	Species*	Antagonist** effect (%)	Similarity (%)	Bacterial load (CFU/g dc)
S1	*Paenibacillus macerans*	61,42[a]	81,6	$7,5x10^3$
S2	*Bacillus circulans*	60,00[a]	82,2	$4,1x10^3$
S3	*Geobacillus thermoglucosidasius*	58,57[b]	90	$2,0x10^3$
S4	*Bacillus licheniformis*	64,28[c]	100	$1,0x10^4$
S5	*Serratia liquefaciens*	58,57[b]	100	$3,3x10^3$
S6	*Bacillus megaterium*	57,14[b]	83,9	$2,7x10^3$
S7	*Paenibacillus glucanolyticus*	52,85[d]	89,3	$1,2x10^3$
S8	*Serratia marescens*	52,85[d]	100	$3,5x10^3$
S9-1	*Pseudomonas aeruginosa*	57,14[b]	100	$1,4x10^2$
S9-2	*Pseudomonas aeruginosa*	60,00[a]	100	$1,7x10^2$
S10	*Sporosarcina pasteurii*	67,14[e]	100	$2,4x10^3$
S11	*Lysinibacillus sphaericus*	52,85[d]	90,1	$3,6x10^3$
S12-1	*Brevibacillus laterosporus*	58,57[b]	94,9	$7,5x10^3$
S12-2	*Brevibacillus laterosporus*	67,14[e]	94,9	$5,5x10^3$
14 strains				

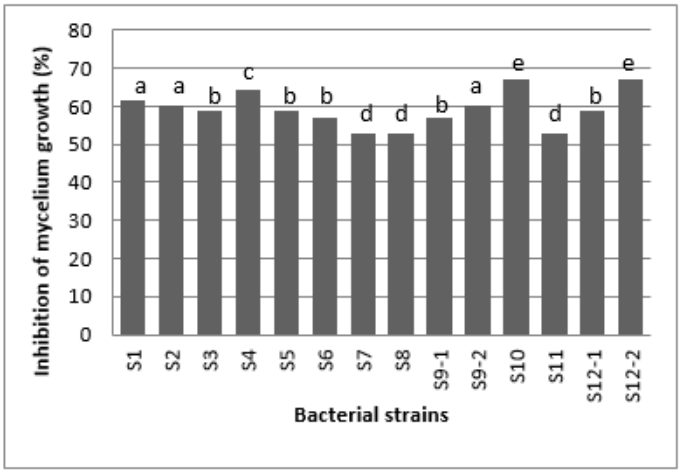
Source: Prepared by the authors (2024)

*Phenotypic identification using Api20E gallery and read by UPBM le lab. 2017-2024 Software on line.

**Antagonist effects (%) sharing the same letters are not significantly different according to Student-Newman-Keuls (SNK) test (at $P \leq 0.05$).

dc= dried compost.

Figure 4: Mean percentage of *in vitro* inhibition of bacterial strains on FORL. Bars sharing the same letters are not significantly different according to Student-Newman-Keuls (SNK) test (at $P \leq 0.05$).



Source: Prepared by the authors (2024)

The abusive use of chemical fungicides to control plants diseases, caused by soil-borne fungi have a negative impact on environment and induce the apparition of resistant strains making them less effective against phytopathogens. The search of new alternative methods to suppress plants diseases without negative effects is necessary today. Several studies showed the effectiveness of compost as a fertilizer and bio-fungicide to suppress a wide range of plants diseases. Its efficacy is related to its microbial community (fungi and bacteria) (Pharand et al., 2002; Hibar et al., 2006; Shanying et al., 2016; Ros et al., 2020; Merah et al., 2022). For that purpose, bacteria from *"Solanum elaeagnifolium" g*reen compost were isolated, identified and then confronted using dual culture (Siameto, 2011) to evaluate their antagonistic activity against one of the most tomato diseases (FCRR) caused by FORL. The antagonistic isolates tested in this study were grouped into eight genera (*Paenibacillus, Bacillus, Geobacillus, Serratia, Pseudomonas, Sporosarcina, Lysinibacillus* and *Brevibacillus*).

The eight genera proved their antagonist activity against FORL, which make them able to be used as biological control agents. In fact, results of the present study are in line with previous studies: Sato et al. (2014) demonstrated the suppressive potentials of *Bacillus* and *Paenibacillus* strains isolated from the tomato phyllosphere against *Fusarium* crown and root rot of tomato. Xinyue et al. (2024) evaluated also that the use of combined agent of *Paenibacillus polymyxa* and *Bacillus cereus* as a potential biocontrol agent to promote the resistance of ginseng to disease, improve the yield, quality and pesticide degradation. Similar results were obtained by Chavez-Díaz et al., (2024), where strains of *Paenibacillus* sp. against *Fusarium* spp. showed its capacities to promote maize growth and inhibit mixed infections of *Fusarium* spp. Elbouazaoui et al. (2022) demonstrated also that strains of *Bacillus, Paenibacillus,* and *Pseudomonas*, represented by different species, isolated from the rhizosphere of healthy chickpea plants growing regions in Morocco, had shown their antagonism against *Fusarium oxysporum* f.sp. *ciceris,* mainly due to the production of diffusible and volatile compounds as well as lytic enzymes.

Among various bacterial antagonists reported, *Bacillus* spp. like *B. licheniformis* have been used for effective control of the diseases by producing a variety of biologically active compounds with a broad spectrum of activities toward phytopathogens and that can induce host systemic resistance (Nakkeeran et al. 2019; Ongena and Jacques 2008). *Bacillus licheniformis* is one of the most popular bacteria in controlling disease either in plant or soil borne. Yan et al. (2020) showed a strong inhibitory effect of *Bacillus licheniformis* isolated from the rhizosphere soil of healthy strawberries on *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *fragariae.* Yadav et al. (2021) showed also a significant inhibitory effect of *Bacillus licheniformis* against *Fusarium* wilt of banana.

Similar effect was observed in case of *Fusarium* wilt of tomato caused by *Fusarium oxysporum* f.sp. *lycopersici* by Karthika et al. (2022).

The antimicrobial activity of *Bacillus circulans* was also studied by many researchers. Mehta et al. (2010) showed that strain of *Bacillus circulans* isolated from apple rhizosphere exhibit a growth inhibition against *Dematophora necatrix* about 46.57% and promoting plant growth. Abada et al. (2014) showed also the antimicrobial and chitinase activity of *Bacillus circulans* against *Fusarium* wilt of cumin and its ability to be used in industry for production of antibacterial compound and in biological control against different plant pathogen.

Members of the genus *Serratia* are also of great research interest because they are almost ubiquitous with antimicrobial properties (Su et al., 2016; Clements et al., 2019). Bharat et al. (2022) evaluated the antifungal potentials of marine bacteria *Serratia marcescens* against eight different *Fusarium* species. Similar results were showed by Michail et al. (2022), where they demonstrated a significant antagonism effect of *Serratia liquefaciens* isolates against *F. oxysporum* by limiting its colony growth. Some strains of genus *Serratia* are able to produce freely diffusible compounds with fungi-static activity *in vitro* confirmed their biocontrol efficacy on fungi pathogens (Michail et al., 2022).

Bacillus thermoglucosidasius are good biocontrol agents too. Wen-Chuan et al. (2011) demonstrated their potential use in the commercial production of lily bulbs, as they can be used alone or in combination with the fungicide Sporgon at low concentration.

Brevibacillus laterosporus proved its use as biocontrol agent against phytopathogenic fungus by many studies. It was showed by Song et al. (2011) a great potential of *Brevibacillus laterosporus* for controlling fungal diseases in apples. This antagonist bacterium was isolated from an apple rhizosphere at Mount Tai in China. The bacterium had strongly inhibited the growth of numerous apple phytopathogens *in vitro*, such as *Rhizoctonia solani*, *Fusarium oxysporum*, *Fusarium solani*, and *Physalospora piricola*. The inhibition rate against the different fungi was ranged from 55.26 to 88.17%. Prasanna et al. (2013) evaluated too the ability of *Brevibacillus laterosporus* to produce enzymes that exhibited antifungal activity against *Fusarium equiseti* and provides evidence for the presence of inducible, extracellular chitinolytic enzymes that contribute to the strain's antifungal and insecticidal activity. Smirnova et al. (2023) proved the use of *Brevibacillus laterosporus* as biological control agent against phytopathogens and insects.

In vitro biocontrol mechanisms of *Lysinibacillus sphaericus*, was also studied by researchers. Shabanamol et al. (2017) showed 100% growth inhibition exhibited by *Lysinibacillus sphaericus* isolated from rice on *Rhizoctonia solani* causal agent of the rice sheath blight via volatile

organic compound production and was positive for the production of siderophores, biosurfactants, hydrogen cyanide (HCN) and ammonia.

For *Bacillus megaterium* Omar et al. (2006) demonstrated its ability to be antagonistic against *Fusarium oxysporum* f.sp. *radicis-lycopersici* causal agent of *Fusarium* crown and root rot in tomato plants. Ehab Saleh et al. (2021) showed also the biocontrol activity of *Bacillus megaterium* against Toxigenic Fungi *Aspergillus flavus, Aspergillus carbonarius, Penicillium verrucosum,* and *Fusarium verticillioides.*

Strains of *Pseudomonas* sp. proved their ability to be good biocontrol agents by several studies. Geoffrey et al. (2010) demonstrated that composts content on fluorescents *Pseudomonas* and especially *P.aeruginosa* had *in vitro* a highest inhibitory effect against *Fusarium* crown of the cucumber under greenhouse by producing antibiotics those suppressed the disease. Sahane et al. (2021) evaluated efficacy of *Pseudomonas* spp. against *Fusarium oxysporum* f. sp. *ciceri.* Owais et al. (2023) proved the use of *Pseudomonas* strains as an alternative to chemical fungicides for management of phytopathogens.

For *Sporosarcina pasteurii* a few studies report its use as biological control against soil-borne phytopathaogens and maybe our results can probably a first report on its inhibitory effect on FORL mycelium growth.

4 CONCLUSION AND PERSPECTIVES

In the present study, we were able to isolate, identify and evaluate *in vitro* 14 potential antagonistic bacterial strains isolated from *"Solanum elaeagnifolium" g*reen compost that can be used in the management of *Fusarium* crown and root rot in tomato plants in Algeria. A large part of the bacterial strains belonging to eight genuses: *Paenibacillus, Bacillus, Geobacillus, Serratia, Pseudomonas, Sporosarcina, Lysinibacillus* and *Brevibacillus.* Two potential antagonist strains (*Sporosarcina pasteurii* and *Brevibacillus laterosporus* (S12-2)) exhibit the highest mycelium growth inhibition of FORL.

Thus, the 14 antagonist bacterial should be tested *in vivo* and under field conditions for future uses as a biological fungicide for FCRR disease management of tomato plants.

Moreover, a future studies will be investigated for more understand of antagonist mechanisms of each species, such as competition for nutrients or iron, competition for infection sites on roots, or production of antibiotics and lytic enzymes.

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