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Screening of agricultural field bacteria and highlighting of their antagonistic effects on post-harvest disease fungi

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ABSTRACT

In this study, the isolation of 374 antagonist bacterial strains was carried out on two agricultural biotopes in which 245 strains were isolated from the soil, 69 from the leaves and finally 60 from the roots of both plants. The screening of the antagonism of these 374 isolates was performed against three phytopathogenic fungi, *Fusarium oxysporum*, *Botrytis cinerea* and *Alternaria alternata*, finding that 24 isolates had antagonistic activity with a mycelial growth inhibition rate equal or higher than 37%. These 24 bacterial isolates were identified on the basis of morphological, physiological and biochemical characters. The comparison with the dual culture technique allowed 6 bacterial isolates (Fr43, F31, Fr52, F101, B6 and B29) to be retained with areas of significant inhibition against *F. oxysporum*, *B. cinerea* and reached a value between 27 and 52 mm. However, these isolates showed significant inhibitory activity against mycelial growth (varies between 25.88% and 67.05%) and sporulation (varies between 68.74 and 100%) of *B. cinerea* and *F. oxysporum* by their supernatant. Concerning the growth promotion, the Fr43 strain was the most active with a GPE % between 25.31 and 60, 71% according to the part of the plant. This isolate was also shown to significantly reduction of the incidence disease to 15.3%, which corresponds to a significant efficacy of biological control that can reach 79.92% against *F. oxysporum*. On the contrary, the isolate F101 exhibited the lowest value of the effectiveness of biological control versus control which can reach only 37.46%.

Key words: Antagonistic, Phytopathogenic fungi, Biological control, Potato.
Article type: Research Article.

INTRODUCTION

The prolonged conservation of crops in survival creates many and varied phytosanitary problems since it is estimated that approximately 20 to 25% of harvested fruits and vegetables are damaged primarily by fungal agents such as *Botrytis cinerea*, *Penicillium expansum*, *Fusarium* sp. during post-harvest handling (Sharma *et al.* 2009). *Botrytis* is regarded as the most important pre-harvest and post-harvest stages fungal pathogen that causes significant losses in fresh fruits, vegetables and ornamentals, (Li Hua *et al.* 2018). In parallel to that, *F. oxysporum* also attacks several crops after harvest and during storage (Abd-Elsalam *et al.* 2007; Al-Enezi *et al.* 2023), which is also responsible for internal browning of stems and roots (Rajput *et al.* 2008). On the other hand, the genus Alternaria, the cause of alternaria, is one of the agents responsible for the rot of fruits such as apples and pears, both in the orchard in conservation (Vorstermans *et al.* 2005). In the past thirty years, there have been extensive research activities to explore and develop strategies based on microbial antagonists to biologically control postharvest pathogens. several antagonists have been identified against the main post-harvest diseases of fruits

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and vegetables such as the strains which belong to species of *Bacillus subtilils* and *Pseudomonas syringae*, species of *Pichia anomala, P. guillermondii, Candida ircofnita, Cryptococcus laurentii* (Spadaro *et al.* 2004; Droby *et al.* 2009; EL-Saman *et al*. 2023; Al-Masoodi *et al*. 2023; Amini *et al.* 2023). In this part, we have isolated the bacterial strains producing antifungal substances by screening them and keeping only those, which are able to show a better activity towards the phytopathogenic species, i.e., *Botrytis cinerea, F. oxysporum* and *Alternaria alternata*.

MATERIALS AND METHODS

Sampling

The samples were collected from the agricultural site which is located at M'nasra, and which is part of the Gharb Basin in Morocco. The samples were taken from two particular fields, the first of which is a strawberry crop of the Splendor variety and the second from a potato field of the Désiré variety. Each sample was represented from healthy plant tissue or symptomatic leaves and roots as well as soil samples from the rhizosphere of two crops. For the soil, the samples were taken at depths of 5 to 10 cm. The samples were taken and then transported to the laboratory and kept at 4 °C for analysis.

Isolation and purification

The bacteria of the plant tissue were isolated by soaking the various pieces in Erlenmeyer flasks, which are containing physiological saline for one hour and were inoculated on LPGA medium. Two grams of each soil sample are suspended in 18 ml of sterile physiological saline. After stirring for 15 min, a series of dilutions of 10^{-1} to 10^{-5} in sterile physiological saline is prepared. 0.1mL of each dilution is inoculated on the surface of the Petri dishes, which are containing the LPGA medium. The incubation is carried out at 26 °C, for 48 hours. From each box, colonies are removed, isolated and then purified. These purified strains are designated by a code number and kept apart.

In vitro antagonist activity on mycelial growth and sporulation

The antagonistic potency of the 374 bacterial isolates which were retained was tested by the inhibition of mycelial growth and sporulation of three phytopathogenic fungi: *F. oxysporum*, *B. cinerea* and *A. alternata*. These isolates belonged to the mycotecus of the Laboratory of the Botany and Protection of plants in Kenitra Faculty of Science. These fungi were cultured on PDA medium at a temperature of 26 ° C while the cultures used for the challenge trials are seven days old.

Direct confrontation

The bacterial suspensions are prepared in the LPG and then adjusted to107 ufc.ml-1. The Petri dishes containing the LPGA medium are covered with 2ml of the bacterial suspension. Then after 15min of incubation, the surplus of the bacterial suspension is aspirated and a 6mm disk of the phytopathogenic mycelium grown on the PDA medium is deposited in the center of the petri dish (Hariprasad *et al.* 2009). After seven days of incubation at 26 °C, the bacterial isolates which gave an inhibition rate greater than or equal to 37% were subjected to extensive antagonistic evaluation tests.

Sporulation

The evaluation of sporulation is done by counting the total number of spores by using a Malassez cell. The values are expressed in number of spores per unit area (mm2) and the percent inhibition of sporulation (Is) is determined by the following formula:

$$\mathbf{Is = (N0 - Nc) / N0 \times 100}$$

where **N0** being the average number of spores which are estimated in the control and **Nc** the average number of spores which are estimated in the presence of the supernatant.

Dual culture technique

The bacterial isolates were cultured on LPG medium at 26 ° C for 24 hours. They are seeded in rectilinear streaks at opposite ends of the agar medium. A obtained cylinder 6 mm in diameter was taken around the perimeter of the cultures of each of the two selected mushrooms and deposited in the center of the petri dish. The results are read

after 7 days of incubation at 26 ° C. The mycelial growth for the two previous antagonism techniques is evaluated by calculating the percentage inhibition according to the following formula:

$$\mathbf{Ic\ (\%) = (D0\text{-}Dc) / D0 \times 100}$$

where D0 is the diametric growth of the control and **Dc** the diametrical growth of the fungus in the presence of a bacterial strain.

Antagonist activity of supernatant on growth and sporulation

For the demonstration of the production of antifungal substances by the bacterial isolates, a bacterial suspension of 48h at 26 ° C is centrifuged at 2500 rpm for 30 min. The LPGA medium is supplemented with the bacterial supernatant which is obtained at 15% and then poured into Petri dishes. Platelets with 6mm in diameter of pathogenic mycelium are deposited in the center of each box. All dishes are incubated at 26 °C for 7 days.

The evaluation of sporulation is done by counting the total number of spores by using a Malassez cell. The values are expressed in number of spores per unit area (mm2) and the percent inhibition of sporulation (Is) is determined by the following formula:

$$\mathbf{Is = (N0 - Nc) / N0 \times 100}$$

where **N0:** being the average number of spores estimated in the control, and **Nc:** the average number of spores estimated in the presence of the supernatant.

Antagonistic activity during potato storage conditions

To continue the evaluation of the criteria of the beneficial strains, bacterization tests were carried out. They consist of protecting potato tubers against Fusarium dry rot during storage. This technique consists in disinfecting potato tubers of the Désirée variety according to the method described by Kim et al (1997). Then they are washed in tap water, disinfected in a disinfected bath for 10 minutes, rinsed with sterile distilled water and finally dried.

The treatment of the tubers is carried out as follows

The tubers are immersed in a spore suspension at 10^7 CFU mL^{-1} for 30 seconds, then dried and stored in a sterile plastic bag. This step is considered as a witness. In the second step, the tubers are first immersed in a bacterial suspension for 30 seconds, dried, coated with a few ml of spore suspension, and finally dried and stored in a sterile plastic bag. Tubers treated in both stages were stored at 4 ºC for 60 days. During the last 15 days, they are incubated at room temperature.

Antagonistic activity IN SITU

Following the in vitro antagonism tests, in situ antagonism tests were performed to verify the efficacy of bacterial isolates in protecting the potato against early *F. oxysporum* attacks and to stimulate growth of the potato. The soil used for in situ tests was taken from an agricultural plot located in the Mnasra region, Morocco and previously autoclaved 121 ºC for 1 h, the operation being repeated 3 times after cooling. The assessment of the ability of bacterial isolates to reduce the incidence of the disease and their effectiveness in biological control. The soil used was sterilized by autoclave and the tubers are disinfected as previously described. The potatoes inoculated by immersion in 9 ml of bacterial suspension at 108 cells / ml are established as follows: some were planted in pots containing a sterile soil, the control receiving a disinfected tuber; and the others were inoculated with 1 ml of spore suspension (10^4 spores mL^{-1}) of *F. oxysporum* before being planted in sterile soil. The control of this last stage receives a tuber treated only with 1ml of spore suspension (10^4 spores mL^{-1}; Elad *et al.* 1985). Subsequently, the second booster was given one week after planting. The pots were placed in a greenhouse and maintained at 23 ºC, 60% humidity and a photoperiod of 12 hours to promote the growth and development of the disease. The plants are regularly irrigated with sterilized water. Each treatment was performed in four repetitions. After six weeks of monitoring the growth of these plants, two results were noted:

- The index of the disease was evaluated using the scale ranging from 0 to 4 (Park *et al*. 2007).

Disease incidence (%) = [(Disease index x number of diseased plant in this index) / (Total number of plants investigated x the highest disease index)] × 100

According to this index, two parameters were estimated as follows (Xue *et al.* 2009):

- Biocontrol efficacy (%) = [(Disease incidence of control - Disease incidence of antagonist treated group) / (Disease incidence of control] × 100.

Evaluation of growth parameters

The potato plants were harvested and their growth was evaluated by measuring the following parameters: fresh material weight (MF), dry matter weight (DM) of the plants after drying at 60 ° C for 72 h, and the height of the plant. In addition, the effectiveness of growth promotion (GPE) was calculated to show the relative effect of antagonistic isolates on plant growth compared to the two control treatments by the following formula (Almoneafy *et al.* 2012):

$$\mathbf{GPE = [(GT - GC) /GC] \times 100}$$

where:

GT: growth parameter in the antagonist group treated, and

GC: growth parameter in the control group.

RESULTS AND DISCUSSION

Screening of antagonistic bacteria

Isolation of antagonistic bacteria

From the leaves, roots and soil of the rhizosphere of two agricultural biotopes we proceeded to the isolation of the bacterial strains by inoculation on the LPGA medium. After an incubation of the isolates at 26 °C for 48 h, the purification results in the constitution of a collection of 374 bacterial strains. A total of 245 bacterial strains were isolated from the soil of the potato plant and strawberry; this is the largest number of isolates obtained in this study. 69 bacterial strains were harvested from the leaves of two plants and 60 strains isolated from their roots.

The preliminary identification of the 374 obtained isolates was performed on the basis of phenotypic methods. This phenotypic study showed that there were four bacterial groups: 120 strains were large Gram-positive bacilli, 34 strains Gram-positive coccobacilli, 180 Gram-negative bacilli and 40 gram-negative cocci (Table 1). Table 1 indicates that all analyzed samples of the two agricultural biotopes show bacteria with varying colonization rates. The distribution of bacterial isolates in both biotopes shows an abundance of bacterial isolates in soil with 245 isolates, while the roots and leaves have only 60 and 69 isolates, respectively. Of the three hundred and seventy-four purified bacterial isolates, 17% were isolated from the potato biotope and 83% from the strawberry biotope. While, high bacterial variability was observed in strawberry soil as potato soil with a colonization rate of 87.75% and 12.24% successively (Fig. 1).

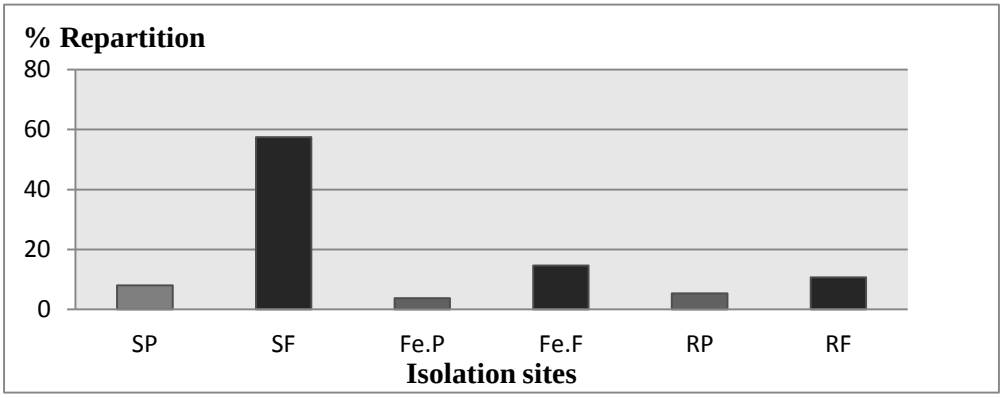

Fig.1. Distribution of bacterial populations in soil, root and leaf of potato plant and strawberry: SP: potato soil, SF: strawberry soil, Fe.P: leaves of apple of soil, Fe.F: strawberry leaves, RP: roots of potato, RF: strawberry roots.

Table 1. Distribution of bacterial isolates by isolation biotopes with some phenotypic characters.

Samples	Sampling site	Number of isolated bacterial strains	Microscopic form	Gram	Total number of isolates
soil	Potato	30	16 Large bacilli	+	120 Large Gram-positive bacilli
			09 Bacilli	-	
			05 Cocci	-	
	Strawberry	215	92 Large bacilli	+	
			98 Bacilli	-	
			10 Coccobacilli	+	
			15 Cocci	-	
Leaves	Potato	14	09 Bacilli	-	34 Gram-positive coccobacilli
			05 Cocci	-	
	Strawberry	55	39 Bacilli	-	
			04 Cocci	-	
			12 Coccobacilli	+	
roots	Potato	20	02 Large bacilli	+	180 Gram-negative bacilli
			10 Bacilli	-	
			02 Coccobacilli	+	
			06 Cocci	-	
	Strawberry	40	10 Large bacilli	+	40 Gram-negative cocci
			15 Bacilli	-	
			10 Coccobacilli	+	
			05 Cocci	-	

Antagonistic effect in vitro on growth and sporulation of phytopatogenic mycelia

After the purification of the isolated colonies, we tested the action of three hundred and seventy-four bacterial strains in vitro by the direct confrontation on growth and sporulation of three phytopathogens: *F. oxysporum*, *B. cinerea* and *A. alternata*. These phytopathogenic isolates come from the mycoteque of the Laboratory of Botany and Plant Protection of the Kenitra Faculty of Science.

Direct confrontation

The direct confrontation of bacterial isolates on the growth of *F. oxysporum*, Botrytis Cinerea and Alternaria Alternata allowed us to retain twenty-four strains with a significant antifungal effect, of which sixteen strains are isolated from the soil, three from the leaves and five from the roots of two agricultural biotopes (strawberry and potato; Table 2). The twenty-four bacterial strains resulted in an inhibition of the growth of the three mycelia with a rate greater than or equal to 37%. While the nine bacterial isolates Fr43, Fr52, F31, F101, Fr45, B6, B33, B29 and P25 were more active with a growth inhibition rate greater than or equal to 60% in the three mycelia. The isolates Fr43, Fr52, F31 and F101 induced a total inhibition of the mycelial growth of *F. oxysporum*. However, the mycelial growth of Botrytis Cinerea was totally inhibited by these bacterial isolates, whereas the Fr52 isolate exhibited a 98.75% inhibition rate of mycelial growth. Total inhibition of *A. Alternata* was induced by isolates Fr43 and F31, while isolates Fr52 and F31 induce a rate of inhibition of mycelial growth of 96.75% and 97% successively (Table 2).

Action on mycelial sporulation

The evaluation of these 24 bacterial isolates on the sporulation of three mycelia allowed us to demonstrate the ability of our isolates to inhibit the sporulation of these mycelia with greater percentages up to the total inhibition of sporulation. However, isolates Fr43, Fr52, F31, F101, Fr45 B6, B33, B29 and P25 represented the highest percentages of sporulation inhibition against the three mycelia (*F. oxysporum, B. cinerea* and *A. alternata*) with an inhibition which varied between 63% and 100% (Table 3).

Mycelial growth of *F. oxysporum*, Botrytis cinerea and *A. alternata* is completely inhibited by bacterial isolates Fr43, Fr52, F31 and F101.

Among the percentage inhibition of mycelial growth greater than or equal to 80%, we noted that the strain Fr45 has a percentage inhibition of 80% and 96% for *F. oxysporum* and Botrytis cinerea successively. Also, bacterial isolate B6 induces a 90.79% inhibition percentage against Botrytis cinerea. In addition, the strains B33 and B29 have a percentage inhibition of 90% and 89% respectively vis-à-vis Alternaria alternate (Table 3).

Table 2. Inhibition rate (in%) of the mycelial growth (Ic) of *Fusarium oxysporum*, Botrytis cinerea and *Alternaria alternata* by the 24 bacterial isolates.

	F. oxysporum	***Botrytis cinerea***	***A. alternata***
	Ic (%)	**Ic (%)**	**Ic (%)**
Control	0 ± 0.0	0 ± 0.0	0 ± 0.0
Fr43	100 ± 0.0	100 ± 0.0	100 ± 0.0
Fr52	100 ± 0.0	98.75 ± 1.8	96.75 ± 1.3
Fr45	72.46 ± 0.6	80.21 ± 0.8	70.49 ± 0.6
Fr42	56.52 ± 0.5	46 ± 1.0	40 ± 0.5
Fr44	53.83 ± 1.2	65 ± 1.0	46 ± 0.5
Fr32	40.46 ± 0.6	51.54 ± 1.5	75 ± 1.5
Fr20	39.50 ± 1.5	55.21 ± 0.8	38 ± 1.0
Fr13	39 ± 1.0	50 ± 2.0	37.68 ± 0.4
Fr12	40 ± 0.8	38.34 ± 0.6	39 ± 2.1
F31	100 ± 0.0	100 ± 0.0	100 ± 0.0
F101	100 ± 0.0	100 ± 0.0	97 ± 0.5
F71	50 ± 1.0	70.56 ± 0.4	52 ± 0.6
F34	35.95 ± 0.3	60.32 ± 0.8	43.87 ± 0.3
F25	39.12 ± 1.4	44.53 ± 0.5	48 ± 1.5
F14	38.65 ± 0.7	41 ± 1.9	42.15 ± 0.9
B6	67.5 ± 1.6	88.38 ± 0.7	61.66 ± 0.2
B33	70 ± 2.0	82.31 ± 0.7	80 ± 2.0
B29	60 ± 1.5	78.06 ± 0.4	75 ± 1.2
P1	48.38 ± 1.1	47.58 ± 1.2	70 ± 1.0
P8	43.54 ± 0.5	50.23 ± 1.2	46.66 ± 0.4
P13	39.51 ± 0.9	40.32 ± 1.5	41.66 ± 0.8
P15	41.93 ± 0.6	48.38 ± 0.4	60.32 ± 0.7
P18	37.09 ± 2.1	51.61 ± 0.8	70.56 ± 0.8
P25	60 ± 1.6	68.06 ± 0.4	71.93 ± 1.0

Confrontation by the dual culture technique

The nine isolates which are retained after the direct confrontation and which are selected by their antifungal power have moved on to determine their mode of antagonistic action through the dual culture technique on two phytopathogenic mycelia (*F. oxysporum* and *B. cinerea*). The results which are recorded in Table 4 and Fig. 2 indeed show that all the tested bacterial isolates inhibited the growth of these pathogens, while they are able to develop zones of inhibition with specific inhibition shapes and diameters. The six bacterial isolates; Fr43, F31, Fr52, F101, B6 and B29 induce zones of inhibition of mycelial growth between 27 and 52 mm, while the isolates Fr45, P25 and B33 have zones of inhibition varying between 3 and 6 mm (Table 4). The production of inhibitory molecules in vitro is the first criterion which is targeted in the research and the screening of potential bacteria agents of biological control. So, the use of the supernatant of six most active bacterial isolates under the dual technique nevertheless confirms their mode of action against phytopathogenic agents.

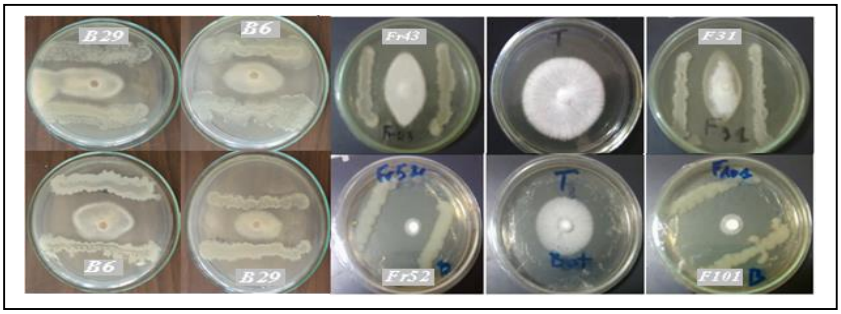

Fig. 2. Antifungal activity by the dual culture of bacterial strains against *B. cinerea* and *F. oxysporum*.

Table 3. Inhibition rate (in %) of the sporulation (Is) of *F. oxysporum*, *B. cinerea* and *A. alternata* by the 24 bacterial isolates.

	F. oxysporum	***B. cinerea***	***A. alternata***
	Is (%)	**Is (%)**	**Is (%)**
Control	0 ± 0.0	0 ± 0.0	0 ± 0.0
Fr43	100 ± 0.0	100 ± 0.0	100 ± 0.0
Fr52	100 ± 0.0	100 ± 0.0	100 ± 0.0
Fr45	80 ± 1.0	96 ± 0.6	72 ± 2.5
Fr42	67 ± 1.4	42 ± 1.0	52 ± 1.4
Fr44	50 ± 2	60 ± 1.7	38 ± 0.9
Fr32	50 ± 2.9	49 ± 1.0	61 ± 1.7
Fr20	38 ± 2.0	40 ± 0.6	40 ± 2.2
Fr13	46 ± 1.0	60 ± 2.1	50 ± 0.7
Fr12	49 ± 2.5	40 ± 2.0	40 ± 1.3
F31	100 ± 0.0	100 ± 0.0	100 ± 0.0
F101	100 ± 0.0	100 ± 0.0	100 ± 0.0
F71	63 ± 1.4	74 ± 0.6	50 ± 2.6
F34	40 ± 0.2	61 ± 1.4	62 ± 2.5
F25	40 ± 1.6	40 ± 1.0	39 ± 1.0
F14	63 ± 2.1	50 ± 3.0	47 ± 2.5
B6	70 ± 0.8	90.79 ± 1.4	74 ± 2.3
B33	73 ± 0.9	70.41 ± 1.6	90 ± 2.5
B29	67 ± 0.3	63 ± 0.8	89 ± 1.8
P1	39 ± 1.0	58 ± 0.6	80 ± 2.6
P8	49 ± 1.2	70 ± 1.6	58 ± 2.5
P13	40 ± 2.1	59 ± 2.0	40 ± 0.8
P15	61 ± 0.7	50 ± 0.8	57 ± 1.0
P18	49 ± 0.9	74 ± 0.3	66 ± 0.8
P25	63 ± 1.4	63 ± 1.8	81 ± 0.8

Table 4. Diameter of the zone of inhibition of mycelial growth (ZI in mm) of *F. oxysporum* and *B. cinerea*.

	Fusarium oxysporum	***Botrytis cinerea***
N° strains	ZI (mm)	ZI (mm)
Fr43	30.00 ± 0.76	32.70 ± 0.42
F31	29.00 ± 1.2	32.20 ± 1.0
F101	31.00 ± 0.8	30.00 ± 0.78
Fr52	27.00 ± 0.32	33.00 ± 0.2
Fr45	3.00 ± 0.6	5.00 ± 0.46
B6	37.50 ± 1.0	37.00 ± 0.14
B33	4.00 ± 1.2	6.00 ± 0.54
B29	52.00 ± 0.6	35.00 ± 0.86
P25	3.00 ± 1.4	4.00 ± 1.14

The effects of supernatants on the growth and the sporulation of the three mycelia

The results which are recorded in Table 5 and Table 6 respectively report the rate of inhibition of mycelial growth and sporulation of F. *oxysporum* and *B. cinerea* after their comparison with the supernatants of six isolates selected by the dual culture technique. The supernatants of six bacterial isolates (Fr43, F31, F101, Fr52, B6 and B29) have the ability to inhibit mycelial growth of *F. oxysporum* with a percent inhibition that ranges from 25.88% to 67.05%. This effect is more important against the mycelial growth of Botrytis cinerea with a percentage inhibition

of the order of 72.54% and 90% (Table 5). The sporulation production of two mycelia was counted in the presence of the supernatant of six bacterial isolates, where percent results of sporulation inhibition are reported in Table 6. In *F. oxysporum*, the percent inhibition of sporulation varies between 68.74 and 100%. The supernatant of isolate F31 is the most active and has a percentage inhibition of 100%. While, sporulation in Botrytis cinerea is totally inhibited by the supernatant of the four bacterial isolates (Fr43, F31, Fr52 and F101), while the supernatant of isolate B6 and B29 has a percentage of the order 82 and 93%. The results of the effect of the supernatants on the growth and the sporulation of three mycelia, nevertheless confirm the mode of action of the selected isolates. The antibiosis phenomenon and more specifically the emission of soluble secondary metabolites are the most probable (Table 6).

Table 5. Antagonist effect of the bacterial supernatant against the mycelial growth of *F. oxysporum and B. cinerea.*

	Fusarium oxysporum	**Botrytis cinerea**
Bacterial isolates	**Ic (%)**	**Ic (%)**
Control	**0 ± 0.0**	**0 ± 0.0**
Fr43	25.88 ± 2.3	88.56 ± 1.4
F31	45.88 ± 1.1	90 ± 2.0
F101	45.88 ± 0.6	83.86 ± 1.6
Fr52	67.05 ± 2.0	81.02 ± 0.8
B6	36.87 ± 3.2	72.54 ± 1.0
B29	40.32 ± 0.6	75.81 ± 1.6

Table 6. Antagonist effect of bacterial supernatant against sporulation of *F. oxysporum and B. cinerea*.

	Fusarium oxysporum	**Botrytis cinerea**
Bacterial isolates	**Is (%)**	**Is (%)**
Control	0± 0.0	0 ± 0.0
Fr43	95.04 ± 1.4	100 ± 0.0
F31	100 ± 0.0	100 ± 0.0
F101	91.89 ± 2.6	100 ± 0.0
Fr52	92.04 ± 3.0	100 ± 0.0
B6	68.74 ± 0.8	82 ± 0.6
B29	70.31 ± 1.2	93 ± 3.0

The antagonistic activities to the storage conditions of potatoes

The protective action of Fusarium dry rot during storage was investigated by coating potato tubers with the fungi in the absence and presence of bacterial suspensions. Fig. 3 illustrates the fact that the bacterial strains Fr43, F31, B6 and B29 are able to protect potato tubers against *Fusarium oxysporum* under storage conditions. This protection results in a complete reduction of the infection rate and the severity of the symptoms caused by *F. oxysporum* compared to the control.

In situ antagonistic activity on the potato

The manipulations of this part consist in verifying the stimulatory and protective effect in situ of four best antagonistic isolates retained in the previous test namely Fr43, F31, B6 and B29 on potato plants.

Evaluation of plant growth stimulation

From the results reported in Table 7 and Fig. 4, we noted that the four antagonistic isolates tested have a significant stimulatory effect on the growth of potato plants. Indeed, the height, the fresh weight and the dry weight of the plants of the potato were significantly improved compared to the control. Consequently, the highest values of all parameters measured against the control were recorded for plants treated with isolate Fr43, B6 and F31 with plant heights of 110.17, 104.01 and 100.68 cm, respectively 52.53%, 44.00% and 39.38% of the GPE test (effectiveness of growth promotion). Regarding fresh weight, we found 100, 77 and 96.1 and 90.05g respectively with a GPE of 60.71, 53.27 and 43.62%. While, the dry weight of the plants was 44.27, 41.9 and 39.03g respectively for strains Fr43, B6 and F31, and their GPE was respectively 25.31, 18.60 and 10.47%. This activity is of less importance in

the B29 strain compared to the control with a rate of effectiveness of the stimulation of the growth of all the measured parameters which are lower than or equal to 19.30%.

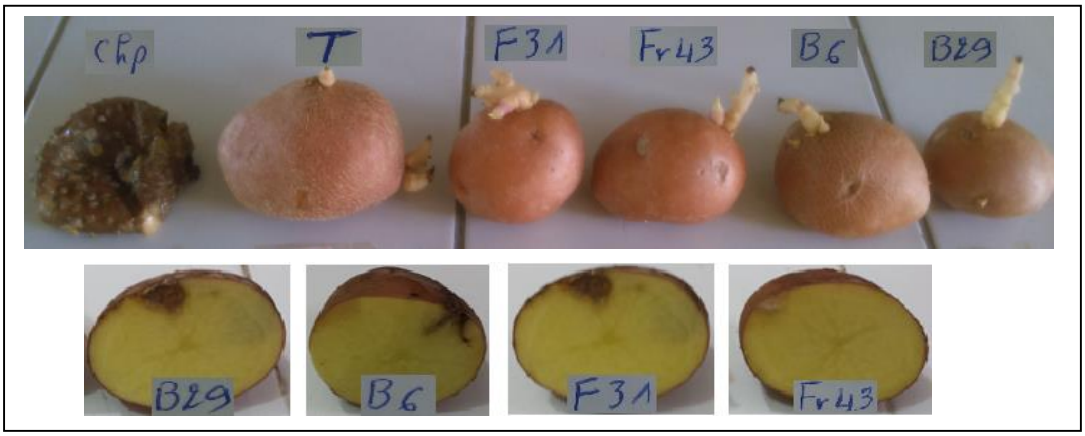

Fig. 3. The ability of bacteria to protect tubers against *F. oxysporum* under storage conditions. **Ch:** control treated with a fungal suspension of *F. oxysporum*, **T:** untreated control, **F31, Fr43, B6, B29:** Tubers treated with bacteria and the fungal suspension of *F. oxysporum*.

Table 7. Effect of four bacterial isolates on height, fresh weight and dry weight of potato plants.

	Height of the plant (cm)		Fresh Weight (g)		Dry weight (g)	
Treatment	**Mean**	**GPE (%)**	**Mean**	**GPE (%)**	**Mean**	**GPE (%)**
Fr43	110.17 ± 0.86 [a]	52.53	100.77 ± 0.71[a]	60.71	44.27 ± 1.27 [a]	25.31
B6	104.01 ± 0.38 [b]	44.00	96.1 ± 0.34 [b]	53.27	41.9 ± 0.64 [b]	18.60
F31	100.68 ± 1.38 [c]	39.38	90.05 ± 1.92 [c]	43.62	39.03 ± 0.88 [c]	10.47
B29	77.78 ± 0.78 [d]	7.68	74.8 ± 0.78 [d]	19.30	37.19 ± 0.75 [d]	5.26
T	72.23 ± 1.05 [g]	-	62.7 ± 1.44 [h]	-	35.33 ± 1.06 [fg]	-

Fig. 4. The effect of antagonistic isolates on growth parameters of potato plants after eight weeks of inoculation.

Reduction of the incidence of the disease by bacterial isolates and their effectiveness in biological control

Table 8 presents the results of the reduction of the disease index and the effectiveness of biological control by our bacterial isolates (F31, Fr43, B6 and B29) according to Park *et al.* (2007). The results shown in Table 8 and Fig.

5 exhibit that all the isolates tested significantly reduce the index of the disease compared to the control. Indeed, the index of the disease for the control plants is 76.31% whereas in the case of the treated plants, it does not exceed 47.71%. As a result, the Fr43 isolate displays a significant reduction in the disease index at 15.3%, as well as its value of the effectiveness of biological control is higher (79.92%) against *F. oxysporum*. However, isolate F31 has the highest disease index and lowest value of biological control efficacy compared to control with 47.71 and 37.46%, respectively.

Table 8. Evaluation of the effectiveness of bacterial isolates on the development of the disease.

Treatments	Disease incidence (%)	Biocontrol efficacy (%)
T	76.31 ± 1.91[a]	-
F.ox+ B6	30.12 ± 0.48 [a]	60.52 ± 1.41[c]
F.ox + F31	47.71 ± 0.79 [b]	37.46 ± 0.61[b]
F.ox + B29	39.96 ± 0.52 [b]	47.63 ± 2.45 [b]
F.ox + Fr43	15.3 ± 0.41 [c]	79.92 ± 0.98 [a]

Note: **F.ox:** *F. oxysporum.* **B6, F31, B29 and Fr43:** bacterial isolates. **T:** control (treatment with *F. oxysporum* alone); The affected averages with the same letter in the same column are not significantly different at the 5% threshold ($p < 0.05$); the data represent the means with four repetitions.

DISCUSSION

In a total of 374 isolates, 245 bacterial strains were isolated from potato and strawberry soil, 69 bacterial strains were also harvested from leaves of two plants and 60 isolated from roots. The results of isolation of bacteria from both agricultural biotopes (potato and strawberry) in this study, show the abundance of bacteria in the soil of both agricultural biotopes relative to different parts of the plants. These results must be interpreted by several biotic and abiotic parameters that are involved in shaping the structure of rhizosphere microbial communities in terms of both diversity and abundance (Dias *et al.* 2013). Among these parameters, the exudations of organic acids (citric, malic, succinic, oxalic and pyruvic), amino acids or lipids by the roots modify the rhizosphere environment (Ben, 2015; Brimecombe *et al.* 2000). Gray and Smith (2005), have also been reported as, the accumulation of different carbonaceous compounds (such as amino acids and sugars) and different chemical signals in the rhizosphere providing a source of energy and nutrients for bacteria. These molecules selectively attract microbial populations capable of metabolizing them, which multiplies preferentially in the rhizosphere. Regarding abiotic parameters, several studies suggest that soil characteristics such as temperature, humidity, pH, and soil texture control the biomass and structure of microbial communities in the rhizosphere (Buyer *et al.* 2010). However, the percentage of bacterial diversity between the two agricultural biotopes (potato and strawberry) was observed in strawberry soil as potato soil. These bacterial diversities are perfectly in line with those reported by Sutra *et al.* (2000), who reported that variability between agricultural sites could be the result of cultural practices and / or the use of chemicals in these agricultural sites. In the same vein of our results, several authors also show that plants would be able to control the bacterial populations of their rhizosphere or even define the taxonomic groups that dominate them (Baudoin *et al.* 2003; Paterson *et al.* 2007; Henry *et al.* 2008). The results of the screening of 370 bacterial isolates from the soils of two agricultural biotopes show that only 7% of strains exhibit antifungal activity. A total of 37% of these show an ability to produce antifungal substance against *F. oxysporum* and *B. cinerea*. These results are consistent with several research studies. Among them, Reghioua *et al.* (2006), show that 11.76% of all actinomycetes isolated from the soil have antifungal activity. The work of Hilali *et al.* (2002), also indicates that, in an initial screening of 85 bacterial isolates, only 18 strains exhibited antifungal activity against *F. culmorum* and *F. graminearum*. In the dual culture, Fr43, F31, B6, and B29 have diffuse zones of mycelial growth inhibition, whereas Fr52 and F101 form well defined mycelial growth inhibition zones. The difference in the development of the zones of inhibition by these antagonistic isolates could be related to the nature and the quantity of the molecules produced in the medium and their modes of action vis-à-vis the pathogen. In this line of research, Siddiqui *et al.* (2000), and other authors have proved that the reduction of fungal growth in vitro by certain bacteria and the formation of zones of inhibition are the result of the release of secondary metabolites. by the bacteria in the culture medium. Montealegre (2003), who reported that fungal growth inhibition without physical contact with antagonistic bacteria, suggests that the isolates could produce extracellular antifungal metabolites. In addition, Ahmed Idris *et al*. (2007) and Zhang *et al.* (2008), reported that among the metabolites there were enzymes secreted by Bacillus spp. that play a role in the lysis of certain phytopathogenic fungi such as *F. oxysporum*, *Rhizoctonia solani*, *Pythium ultimum* and *Alternaria solani*. The diameters of the zones of inhibition that we observed in our work are greater than or equal to 27 mm, which is in

agreement with Xu *et al.* (1986), which clearly showed that a zone of inhibition greater than or equal to 13 mm is a very good criterion for in vitro isolation of antagonistic bacteria. The results obtained by the dual culture technique in our work reveal the importance of our isolates selected as antagonistic agents with different modes of action. These results are in agreement with several authors who have also used this technique and selected antagonistic strains. Among which are Karimi *et al*. (2012) who selected *Pseudomanas fluorescence* antagonistic agents against *Clavibacter michiganensis*. Hmissi *et al.* (2011) also selected Rhizobium isolates antagonistic to *Fusarium culmorum* responsible for wheat crown rot. The supernatant challenge of the six bacterial isolates showed antifungal activity against growth and sporulation of both mycelia. The inhibition rate of these parameters varies between 23% and 100%, with more effective antagonism against Botrytis cinerea than *F. oxysporum*. These results nevertheless confirm the mode of action of the selected isolates. The antibiosis phenomenon is more precisely the emission of soluble secondary metabolites is most likely. These results are in agreement with those of Abdulkareem *et al.* (2014) who showed that Bacillus subtilis fundamentally reduced the growth of the pathogen by the secretion of diffuse inhibitory substances. Ardebili *et al.* (2011) also demonstrated that P. fluorescens can inhibit the growth of *F. oxysporum* by secretion of enzymes. Following our in vitro study which allowed us to retain six bacterial strains with significant antifungal activity, we evaluated the different characteristics of a PGPR agent of four most active bacterial isolates, it is the strain B6, F31, Fr43, and B29. Among the tests is the protection of potato tubers against dry rot during storage. The tuber coating antagonism test demonstrated the protective capacity of the four bacterial isolates B6, F31, Fr43, and B29 with respect to *F. oxysporum*. This protection resulted in a total reduction of Fusarium dry rot and symptom severity compared to the control. These results are in agreement with those of Recep *et al*. (2009), who selected three bacterial strains with Fusarium dry rot inhibition capacity when they were tested against the three strains of *Fusarium sambucinum, F. oxysporum* and *F. culmorum* in one test on potato tubers. These strains of the genus *Pseudomonas, Enterobacter* and *Pantoea*. Slininger *et al*. (2007), also showed that *P. fluorescens* and *Enterobacter cloacae* have the ability to inhibit late blight and pink rot of potato under storage conditions. Regarding the determination of stimulatory and protective activity in situ, we noted that the four antagonistic isolates tested have a significant stimulatory effect on plant growth, with higher values for all measured parameters compared to the control. These results are consistent with previous research, including the work of Orhan et al. (2006), who have shown that Pseudomonas BA-8 and Bacillus OSU-142 strains alone or in combination increase the nutrition, growth and yield of cherry plants. Previously, Gholami *et al.* (2009), prospecting for PGPR (Plant Growth Promoting Rhizobacteria) in sterile and non-sterile soils, found that six bacterial isolates significantly increased the dry weight of leaves and shoots and the leaf surface. Antagonism tests in situ against Fusarium wilt have also demonstrated an action similar to that of the coating. This action resulted in reduced incidence of Fusarium disease and stimulation of the parameters of potato growth. This result may be due to antibiosis or the production of plant growth factors or both. Indole-3-acetic acid is the most common phytohormone produced by antagonistic bacteria. These results are in agreement with those of Igarashi (2002), Dobbelaere (2003) who recently proved that several species of the genera *Pseudomonas, Bacillus, Paenibacillus, Rhodobacter, Azospirillum* and *Streptomyces* secrete the three indole-acetic (IAA) when fed with L-tryptophan, which improves plant growth. However, the main source of tryptophan in soil is exudates from plant roots (Spaepen *et al.* 2007). Results similar to our potato study were obtained by Aliye *et al.* (2008), which reported the antagonistic activity of P. fluorescens PF20 and PF9 in vivo in sterile soil. In fact, these bacteria are at the origin of the reduction of wilting, the increase of the biomass of the potato plants, and the significant reduction of the disturbances of the pathogen. In general, beneficial bacteria protect the plant through three major mechanisms that are rhizocompetence, antibiosis and stimulation of plant defense. Rhizocompetence implies that bacterial isolates have the capacity to effectively colonize the root and become well adapted to the use of compounds exuded by the plant (sugars, carboxylic acids, certain amino acids ...). Moreover, a competition for trophic resources also explains an important adaptation and this is studied for the competition for iron resources thanks to a specific system of siderophores (Cornelis *et al.* 2002). Antibiosis in turn involves mainly the synthesis of antimicrobial compounds by our bacterial isolates such as phenazines and pyoluteolin, on the other hand, can secrete certain volatile compounds such as HCN which also plays a role in the protection of the plant against phytopathogens.

Finally, the stimulation of plant defense is mainly due to signaling pathways. Among them, the SAR pathway whose signal molecule is salicylic acid. In the same vein, Maurhofer *et al.* (Maurhofer *et al*. 1998), has shown in some plant / pathogen models that salicylic acid is exogenously supplied by fluorescent Pseudomonas and conferred protection against pathogens. By comparing the results obtained in vitro and in situ of our bacterial

isolates, it appears that there is a correlation between them. According to the literature, good in vitro results can not always be reproduced reliably under field conditions. The variability in the performance of PGPR may be due to a variety of environmental factors such as plants age, species, specific genotypes, and root exudates are that may affect their growth and exert their effects on plants (Li *et al.* 2006). Environmental factors include the climate, weather, characteristics and activity of the native microbial flora of the soil.

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