

Evaluation of the Effect of Four Bacterial Isolates on the Plant Growth Promoting and the Reduction of Dry Rot of Potatoes

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ABSTRACT: The purpose of the following study is to evaluate the potential of four bacterial antagonists to reduce the development of the *Fusarium* dry rot and evaluate their role in growth promotion efficacy (plant height, fresh weight and dry weight) of the potato.

After the screening in vitro, four antagonistic strains caused an inhibition of the fungi with a diameter greater than 27 mm, namely Fr43, Fr52, F101 of *Pseudomonas sp* gender, and F31 of *Acinetobacter* gender. These strains were selected to study their effect in vivo.

The treatment of tubers by successive immersions in the spores and in the bacterial suspensions showed the ability of the latter to protect the tubers during the storage.

Concerning the growth promotion, all the applied antagonistic isolates exhibit a significant difference compared to the control. Consequently, the Fr43 strain is the most active. It has a height of 110.17cm, a fresh weight of 100.77cm and a dry weight of 44.27cm with a respective correspondence of 52.53%, 60.71 and 25.31% of the test GPE%.

For the reduction of the Disease incidence, the isolate Fr43 also demonstrated a significant reduction in the incidence of the disease with 15.3%, which corresponds to a significant effectiveness of biological control of up to 79.92% against *Fusarium oxysporum*. On the contrary, the isolate F101 has the lowest value of the effectiveness of biological control versus control which can reach only 15.7%.

Consequently, the isolates Fr43, Fr52 and F31 have a potential use not only in the bio-protection of potato but also in the integrated management of *Fusarium* wilt. They are also considered as PGPR.

KEYWORDS: Antagonistic effect, *Fusarium oxysporum*, potato, PGPR, biological control.

I. INTRODUCTION

Fusarium dry rot is a disease that strikes potatoes and it is well-known all over the world. It is caused by several species of *Fusarium spp*. This fungus can be transmitted from infected seeds to the sound ones during its harvest or even its handling. After its harvest, *Fusarium* can cause dry rot in warehouses, which reduces the quality of production.

The dry rot fungus is generally found on seed tubers, and also has the ability to survive in the soil for a long period of time. Traditionally, the management of these diseases has been performed with fungicides such as thiabendazole. However, the resistance to thiabendazole in isolates of *Fusarium* has been reported in Europe, the USA and Canada [8, 14].

Biological control is thus being considered as an alternative or a supplemental way of reducing the use of chemicals in agriculture [3, 15, 23]. The use of biological control agents in the management of fruits and vegetables diseases in post-harvest was reported to be more effective and feasible [10; 16].

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In this situation, the research raises new and effective methods of controlling the dry rot of potato. Eighteen bacterial strains belonging to the genera *Pseudomonas*, *Enterobacter* and *Pantoea* are consistently responsible to inhibit the dry rot of potato vis-à-vis three different strains of *F. sambucinum* [17].

This chapter aims partly at assessing the antagonistic power of the three strains of *Pseudomonas* and one *Enterobacter* vis-à-vis the *Fusarium spp* (causative agent of dry rot of potato) and partly testing the effects of these bacterial strains to stimulate growth of the potato plant.

II. MATERIALS AND METHODS

1. Materials

Based on the tests of microbiological characterization and screening criteria of the beneficial strains, in particular, the antagonistic activity in vitro, four strains of the genus *Acinetobacter* and *Pseudomonas* sp (Fr43, Fr52, F101 and F31) were selected from a collection of 310 bacterial strains. These strains showed significant powers in the action of biocontrol in vitro [7].

The *Fusarium oxysporum* used in this study comes from the Laboratory of Botany and Plant Protection, Morocco Kenitra [24].

2. Tuber coating technique

This technique involves the disinfection the variety of potatoes tubers variety “Desiree” according to the method described by [11]. Then they are washed with tap water, disinfected in a bath for a time of 10 min, rinsed with sterile distilled water and finally dried.

The treatment of tubers is performed as follows:

- Tubers are immersed in a spore suspension of 10^4 spore / ml for 30 seconds, then dried and stored in a sterile plastic bag. This step is considered as the control.

- 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 at the end dried and stored in a sterile plastic bag.

The tubers treated in two stages were stored at 4 ° C for 60 days. During the last 15 days, they were incubated at room temperature.

3. Tests of antagonism in situ

After the tests of antagonism in vitro, the tests of antagonism in situ were conducted to verify the effectiveness of bacterial isolates to protect the potato against early attacks of *Fusarium oxysporum* and growth promotion efficacy of the potato.

The used soil was sterilized and the tubers were disinfected as described above. The potatoes inoculated by immersion in 9 ml of bacterial suspension (Fr43, Fr52, F101 and F31) of 10^8 cells / ml were as follows: some were planted in pots containing sterile soil, and the control receives a disinfected tuber; the others were inoculated into 1 ml of spore suspension (10^4 spores / ml) of *Fusarium oxysporum* before being planted into the sterile soil. The control of this final stage receives a tuber treated solely with spore suspension of 1 ml (10^4 spores / ml) [22]. Subsequently, the second point of the antagonist was performed one week after the plantation.

The pots were placed in a greenhouse and maintained at 23, 60% of humidity and a photoperiod of 12 hours in order to promote the growth and development of the disease. Plants were regularly irrigated with sterile water.

Each treatment was performed in four replicates. All treatments were distributed in a randomized position to avoid any positional effect on plant growth.

4. Notation results

The potato plants were monitored for the development of the disease for eight weeks. Disease incidence was evaluated using the scale varying from 0 to 4 [13]. According to this index, two parameters were estimated as follows [21]:

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$$\text{Disease incidence (\%)} = \frac{(\text{Disease index} \times \text{number of diseased plant in this index})}{(\text{Total number of plants investigated} \times \text{the highest disease index})} \times 100$$

$$\text{Biocontrol efficacy (\%)} = \frac{[(\text{Disease incidence of control} - \text{Disease incidence of antagonist treated group}) / (\text{Disease incidence of control})] \times 100.}$$

5. The evaluation of the growth parameters

The plants of potato were harvested and their growth was evaluated by measuring the following parameters: weight of the fresh material, the weight of dry matter of plants after drying at 60 ° C for 72 h and the height of the plant. In addition, growth promotion efficacy (GPE) was calculated to show the relative effect of the antagonistic isolates on plant growth compared to the two control treatments by the following formula [2]:

GPE (%) = [(GT - GC) / GC] x 100; GT; growth parameter in the treated antagonist group, and GC; growth parameter in the control group.

6. Statistical analysis:

The GenStat software was used for data analysis. Mean comparisons were conducted using a Least Significant Difference (LSD) test (P < 0, 05).

III. Results

Following our previous study that allowed us to retain four bacterial strains having a strong antifungal activity including Fr43, Fr52, F101 of *Pseudomonas sp* gender, and F31 of *Acinetobacter* gender [7], we will demonstrate the ability of these antagonistic isolates to protect the tubers of dry rot during the storage, growth promotion efficacy of the potato and the effectiveness of biological control.

The protective action of *Fusarium dry rot* in storage was tested by coating the potato tubers with mycelium in both the absence and the presence of the bacterial suspensions. Figure 1 shows that the bacterial strains Fr41, Fr52, F101 and F 31 are able to protect potato tubers against *Fusarium oxysporum*.



Figure 1: the ability of bacteria is protecting the tubers against *Fusarium oxysporum* in the storage.
Ch: control treated with a fungal suspension of *Fusarium oxysporum*, **T:** untreated control, Fr52, Fr43, F101, F31: bacteria and the fungal suspension of *Fusarium oxysporum*.

1. Growth promotion

Table 1: Effect of application of antagonistic bacterial isolates and *Fusarium oxysporum* on the height and weight of fresh and dried potato plants

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Mean followed by the same letter within a column are not significantly different as determined by the LSD test ($p < 0.05$).

According to the results shown in table 1 and figure 2, we noted that all the antagonistic isolates applied in the stimulation of growth show a significant difference between the treatments concerning the height, fresh weight and dry weight of potato. Accordingly, the highest values of all the measured parameters compared to the control were recorded in the treated plants with the isolate Fr43, Fr52 and F31 whose height was 110.17cm, 103.9cm, and 100.68cm respectively, with a correspondence of 52.53%, 43.84% and 39.38% of the test GPE%. Concerning the fresh weight, we respectively found 100.77g, 95.6g and 90.05g with a PEG of 60.71%, 52.47% and 43.62%. On the contrary, the plants' dry weight was 44.27 g, 42.5g and 39.03g respectively in the strains Fr43, Fr52 and F31, and their GPE was respectively 25.31%, 20.30% and 10.47%.

Figure 2: Effects of antagonistic bacteria on the growth of potato plants of after eight weeks of inoculation



treatment	Plant's height (cm)		fresh weight (g)		dry weight (g)	
	Mean	GPE (%)	Mean	GPE (%)	Mean	GPE (%)
Fr43	110,17±0,86a	52,53	100,77±0,71a	60,71	44,27±1,27a	25,31
Fr52	103,9±0,38b	43,84	95,6±0,41b	52,47	42,5±0,55b	20,30
F31	100,68±1,38c	39,38	90,05±1,92c	43,62	39,03±0,88c	10,47
F101	78,73±0,78d	8,99	75,4±0,94d	20,25	38,13±0,97d	7,92
T	72,23±1,05g	—	62,7±1,44h	—	35,33±1,06fg	—

Table 2: Disease incidence and biocontrol efficacy of treatments with four isolates Fr52, Fr43, F101 and F31 against *Fusarium oxysporum*

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2.The evaluation of the disease

Mean followed by the same letter within a column are not significantly different as determined by the LSD test ($p < 0.05$); the data represent the average of the experiment with four replicates each.

Table 2 show that all the antagonistic isolates are significantly different compared to the control. Consequently, the Fr43 isolate presents a significant reduction in the incidence of the disease at 15.3%, and its value of the Biocontrol efficacy is higher up to 79.92% against *Fusarium oxysporum*. However, the F101 isolate has the highest incidence of the disease and the lowest value of the Biocontrol efficacy compared to the control is respectively with 64.31 % and 15.7%.

Treatment	Disease incidence (%)	Biocontrol efficacy (%)
T	76,31±1,91a	-
Fs+ F101	64,31±0,79a	15,7 ± 1.29c
Fs+ F31	47,71±0,79b	37.46 ± 0.61b
Fs+ Fr52	51,46±0,67b	32,51 ± 2,45b
Fs+ Fr43	15,3±0,41c	79,92 ± 0,98a

Figure 3: Effect of bacterial isolates on disease development



IV. DISCUSSION

The testing of antagonism with tubers' coating showed the protective capacity of *pseudomons sp* (FR52, FR41, and F101) and *Acinetobacter* (F31) towards the *Fusarium oxysporum*. This protection has resulted in the reduction of *Fusarium* dry rot and symptoms' severity compared to the control. These results are in agreement with [18], who have selected three bacterial strains of the genus *Pseudomonas*, *Enterobacter* and *Pantoea*. The latter have the inhibitory capacity of the dry rot *Fusarium* when they were tested against the three strains of *Fusarium sambucinum* in a test on the potato tuber.

Slininger & al.[19] also showed that the *Enterobacter Cloacae* and *Pseudomonas fluorescens* has the power to inhibit the late blight and the pink rot of potatoes in the storage conditions. Moreover, the tests of antagonism of these four strains in situ towards *Fusarium oxysporum* have also demonstrated a similar effect to that of the coating of the tubers.

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This action resulted in reducing the Disease incidence of the plants and the Biocontrol efficacy. Moreover, the plants treated with these four strains showed significantly higher values of the height and the bio-mass of potato plants (fresh and dry weight) compared to the control except the F101 strain that has a less significant effect compared to the control.

Some previous works are in agreement with our study including Aliye & al. [1] who reported the antagonist activity of *P. fluorescens* PF20 and PF9 in vivo into sterile soil. Indeed, these bacteria are causing the reduction of wilting, increasing the biomass of potato plants, and reducing significantly the pathogenic disturbances.

Some earlier studies have also reported that actinomycetes and fluorescent *Pseudomonas* spp show an antagonistic activity against bacteria and phytopathogens plants [4, 6, 5]. This antagonist activity may be due to the antibiosis or the production of factors of plant growth, or both. This result is in agreement with that of Manulis [12] and Igarashi [9] who has shown that several species of *Streptomyces* secrete the three-indole acetic acid (IAA) once fed with L-tryptophan, which improves the plant growth.

V. GENERAL CONCLUSION

From these results we can conclude that the four tested bacterial strains have a potential in protecting and stimulating the growth of the potato in greenhouse conditions. However, further testing is still necessary to study their mode of action and confirm their effectiveness in open air conditions

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