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Biological Control of Atypical Pink Rot Disease of Potato in Tunisia

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ABSTRACT

In order to biologically manage the atypical pink rot disease of potato, various indigenous antagonistic bacteria were tested against *Pythium aphanidermatum*. *In vitro* tests showed that *Achromobacter xylosoxidans* (B_4), *Pseudomonas putida* (B_7) and *P. fluorescens* (B_{10}) isolates resulted in significant decreases in the mycelial growth of *P. aphanidermatum* ranging from 50 to 88%. Therefore, they were further evaluated for their ability to produce diffusible metabolites in culture filtrates. *P. fluorescens* was shown to be the most efficient by exhibiting the highest inhibitory activity *in vitro*. Total inhibition of *P. aphanidermatum* growth was achieved with the culture filtrates of this antagonistic agent applied at a 1/2 (v/v) ratio. The biological treatments of inoculated potato (*Solanum tuberosum* L.) tubers cv. 'Spunta' by the bacterial culture filtrates were also efficient in reducing the rotting severity by more than 90% with *P. fluorescens* as compared to the untreated and inoculated tubers. Thus, the management of natural potato infection in storage and in the field by applying some antagonistic bacteria should be investigated.

Keywords: antagonists, culture filtrate, growth inhibition, rot severity, *Solanum tuberosum* L., tuber pink rot
Abbreviations: BCA, biological control agent; **CA**, carrot agar; **CMA**, corn meal agar; **KB**, King's medium B; **LB**, Luria Bertani agar medium; **OMA**, oatmeal agar; **PDA**, potato dextrose agar medium

INTRODUCTION

In Tunisia, potato (*Solanum tuberosum* L.) tuber losses in storage were frequently attributed to a number of damaging attacks of soil-borne pathogens which may be involved in the most serious post-harvest diseases such as dry rot caused by various *Fusarium* species and wet rots induced by several *fungus-like* and bacterial pathogens such as *Phytophthora* spp., *Pythium* spp. and *Pectobacterium* spp. (Priou and El Mahjoub 1994; Daami-Remadi 1995; Priou and French 1997; Priou and El Mahjoub 1999; Triki *et al*. 2001a, 2001b; Daami-Remadi *et al*. 2006). In Tunisia, *Pythium aphanidermatum* and *P. ultimum* were isolated from diseased potato tubers exhibiting rots in rustic summer as well as in refrigerated stores. This disease is one of the most important diseases affecting potato tubers in the field and during the traditional storage (Priou and El Mahjoub 1994; Priou and French 1997; Priou and El Mahjoub 1999; Triki *et al.* 2001a, 2001b). Although this pathogen can be seed transmitted (Priou and El Mahjoub 1994), it was frequently isolated from soil (Priou *et al*. 1997; Triki *et al*. 2001b). Symptoms shared features described for both pink rot, caused by *Phytophthora erythroseptica*, and leak, caused by *Pythium* spp. Infected tubers were shaded brown, with a black line, sometimes barely visible through the tuber skin. Black discoloration of tissues occurred beneath the lenticels but there was no internal advancing black margin in cut tubers, although the flesh turned pink in a few minutes, and then black. Tubers were spongy and remained intact, exuding a large amount of clear, odourless liquid.

The methods currently employed for the control pink rot and leak of potato are mainly based on prophylactic measures by using pathogen-free tubers (Daami-Remadi *et al*. 2001b; Triki *et al.* 2001a, 2001b) and metalaxyl based fungicides. This chemical treatment was considered to be the most common agent of emergence of tuber pink rot (Mulrooney 1982; Torres *et al*. 1985; Roy *et al.* 1992; Zinc 1995). Moreover, Trapero-Casas *et al*. (1990) have successfully controlled the pre-emergence damping-off of chickpea caused by *Pythium* sp. by seed treatment with metalaxyl. However, the use of chemical fungicides has been shown frequently inefficient, to be costly and toxic. Thus, there is renewed interest in biological control based on the application of antagonistic micro-organisms which are known to act as biological control agents (BCAs) for the suppression of plant pathogens. Weller *et al.* (2002) has considered the use of fluorescent *Pseudomonas* spp. and *Trichoderma* spp., two groups of BCAs, for the natural suppression of various herbaceous soil-borne pathogens. Weller *et al.* (1988), Callan *et al*. (1990), and Hultberg *et al*. (2000) reported that *Pseudomonas* sp. isolates represent potent BCAs against *P. ultimum* the causal agent of corn and tomato damping-off, respectively.

The present study was undertaken to investigate the potential use of bacterial isolates from the soil for the biological control of *P. aphanidermatum* and *P. ultimum*.

MATERIALS AND METHODS

Tuber pathogenic fungi

Pythium strains were isolated from diseased tubers, stems and field soils from different regions where severe losses were observed. They were brought separately to the laboratory for diagnosis. Sam-

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ples showing symptoms were rinsed twice in sterilized distilled water then disinfected with alcohol 95%. Small fragments were cut aseptically and were placed on plates containing oatmeal agar (OMA, Difco) supplemented with streptomycin sulphate (300 mg/l) and incubated at 25°C for three days. Identifications were based on culture morphology and microscopic characteristics of sexual organs produced on carrot agar (CA) medium when incubated for five days at 25°C. CA is rather transparent as compared to OMA medium and allows direct microscopic observations. Sporangia were produced by using a suspension of sterilized horticultural compost in distilled water and then compared with microscopic observations (Triki *et al*. 2001).

Preliminary pathogenicity tests were carried out according to previously described protocols (Triki *et al*. 2001a) where tubers cv. 'Spunta' were inoculated by *Pythium* spp. isolates and subsequently submitted to incubation for detection of typical symptoms of atypical pink rot. The preliminary tests indicated that all the strains of *Pythium* spp. were pathogenic to potato. The optimum temperatures for infection were 30°C for *P. aphanidermatum* and 25°C for *P. ultimum*, corresponding to the optimum temperatures required for their *in vitro* growth. For all the biological control assays we used only the isolate *P. aphanidermatum*.

Isolation and identification of antagonistic bacteria

Putative antagonists used in this work were obtained from the collection of Plant Protection Laboratory of the Olive Tree Institute of Tunisia. They were isolated from Tunisian soils, from compost juice and from the rhizosphere of Saharan plants. They were streaked on Luria Bertani (LB) agar medium plates [containing (g/l): yeast extract 5, peptone 10, NaCl 10 and bacteriological agar 20] and incubated at 26°C for 3 to 5 days. Purified colonies were stored in 30% glycerol solution at -20°C.

Identification of the antagonistic strains used in this study were made by sequencing the 16S rRNA gene (*rrs*) according to the method described in Triki *et al.* (2012).

In vitro screening of antagonistic activity

1. Dual culture

The *in vitro* inhibition of the mycelial growth of *Pythium* strains by the bacterial isolates was tested using the dual culture method as previously described by Landa *et al*. (1997). A 10 µl of an antagonistic bacterial suspension (10^8 CFU/ml) was placed on the margin of a potato dextrose medium (PDA) and incubated at 25°C for 24 h. A 4-mm agar disc from fresh CMA cultures of *Pythium* spp. was placed at the centre of the PDA plate for each of the bacterial isolates tested and incubated at 25°C for 3 days.

2. Antifungal activity of diffusible metabolites

For this test, only the bacterial filtrates of the strains B_4, B_6, B_7, B_{10}, B_{14} and B_{19} were assayed for their ability to produce diffusible extracellular metabolites using the diffusion method. All bacterial isolates were transferred individually to 50 ml of LB broth medium in 250 ml Erlenmeyer flasks and the bacterial cultures were incubated by shaking at 200 rpm for 3 days at 25°C (Ben Ameur 2006). They were then centrifuged at 3000 rpm for 20 min to remove cell debris. After that, the prepared solutions of each sample were filtered through 0.22 µm filters (Sterivex-GS filter units) under sterile conditions and were added to a PDA medium at 1/6, 1/2, 1/1, 2/1 v/v ratios (Lemriss *et al.* 2003) (**Table 1**). For the control plates, only water distilled water was added at the same ratios. Then, a plug of *P. aphanidermatum* (3 mm in diameter) was deposited laterally on PDA plates. The plates were incubated at 25°C for 3 days, until the mycelium growth of *P. aphanidermatum* of the untreated control reached the periphery of the Petri dishes.

For *in vitro* tests, the diameters of the fungal colonies were measured and compared to that of the control for which the bacterial suspension was substituted by sterile distilled water. Each experiment was performed in triplicate and repeated three times. The percentage of growth inhibition was calculated using the following formula:

% inhibition = [1-(treated fungal growth/control growth)] ×100.

Table 1 Ratios (v/v) of antagonistic bacterial filtrates added to PDA medium.

Medium/Ratios	$D_{1/6}$ (v/v)	$D_{1/4}$ (v/v)	$D_{1/2}$ (v/v)	$D_{1/1}$ (v/v)	$D_{2/1}$ (v/v)
Supernatant of antagonistic bacteria	0.7 ml	1 ml	1.7 ml	2.5 ml	3.3 ml
PDA	4.3 ml	4 ml	3.3 ml	2.5 ml	1.7 ml

In vivo assays against *Pythium* spp. tuber rot

To evaluate the efficiency of bacterial antagonistic agents tested in controlling tuber rotting, oospores suspensions of *P. aphanidermatum* and cell suspensions of each bacterial isolate were prepared. The selected bacterial strains were incubated in broth media (LB or KB) (KB: King's medium B) under continuous agitation at 25°C for 3 days before use. Then, the bacterial suspensions were adjusted at 10^8 CFU/ml. The cells were harvested by centrifugation for 20 min at 3000 rpm and the prepared suspensions of each bacterial supernatant was filtered through 0.22 µm filters under sterile conditions. The concentrations used for oospore suspensions were adjusted to 10^3 oospores/ml using a Malassez cytometer (Witeg Labortechnik GmbH, Germany). Oospores were collected from *P. aphanidermatum* colonies grown on corn meal agar (CMA) medium for 10 days at 30°C. In order to remove saprophytes and soil, ten tubers cv. 'Spunta' was disinfected with 10% (dw/v) sodium hypochlorite solution for 30 min and then rinsed three times with sterile distilled water Inoculation was made by deposing 100 µl of the oospore suspension (10^3 oospores/ml) in cylindrical wound (0.5 cm × 0.5 cm) made by a sterile cork borer. Treatments were applied by deposing 100 µl of the filtrate solution for each bacterial isolate in the occasioned wounds. Preventive treatments were made 12 h before tuber inoculation and the curative ones were realized 12 h after challenge with pathogen. Untreated control tubers were treated with 100 µl of sterile distilled water. The treated tubers were incubated for 3 days at 25°C and data were recorded as described previously by Triki *et al.* (2001b). Tubers were cut across the inoculation sites to measure the necrosis width (W, mm) and the necrosis depth (D, mm) from the surface along the inoculated wound and in order to compute the penetration (P, mm) of tissues following the formula described by Lapwood *et al*. (1984): P = (W/2 + (D-5)) / 2. Data were recorded for the hole per tuber and the mean penetration was calculated for each tuber. Tubers were sampled as replicates.

Statistical analysis

The data were subjected to analysis of variance using the Statistical Package for the Social Sciences (SPSS V.11; SPSS Inc., Chicago, IL). Mean values among treatments were compared by Duncan's multiple range test at the 5% ($P \leq 0.05$) level of significance.

RESULTS

Isolation of microorganisms and screening of potential antagonists

The inhibitory spectrum of thirty bacterial isolates, obtained from Tunisian soils, compost juice or the rhizosphere of Saharan plants evaluated against *P. aphanidermatum*, revealed that 12 isolates (B_1, B_3, B_4, B_5, B_6, B_7, B_{10}, B_{12}, B_{14} and B_{19}) were able to inhibit the pathogen growth by agar diffusion assay.

The biochemical and the molecular methods allowed the identification of the antagonistic bacteria as *Burkholderia cepacia* (B_1), *Bacillus megaterium* (B_3), *Achromobacter xylosoxidans* (B_4), *Bacillus thuringiensis* (B_5), *Pseudomonas* sp. (B_6), *P. putida* (B_7), *P. fluorescens* (B_{10}), *Pseudomonas* sp. (B_{12}), *B. subtilis* (B_{14}) and *P. pseudoalcaligenes* (B_{19}).

All the antagonistic bacteria tested against *P. aphanidermatum* had significantly reduced the pathogen mycelial growth (**Fig. 1**). The highest growth inhibition percentages ranging from 50 to 88% were obtained with the isolates B_4, B_7, and B_{10} (**Fig. 1A, 1B**). With the remaining isolates (B_1,

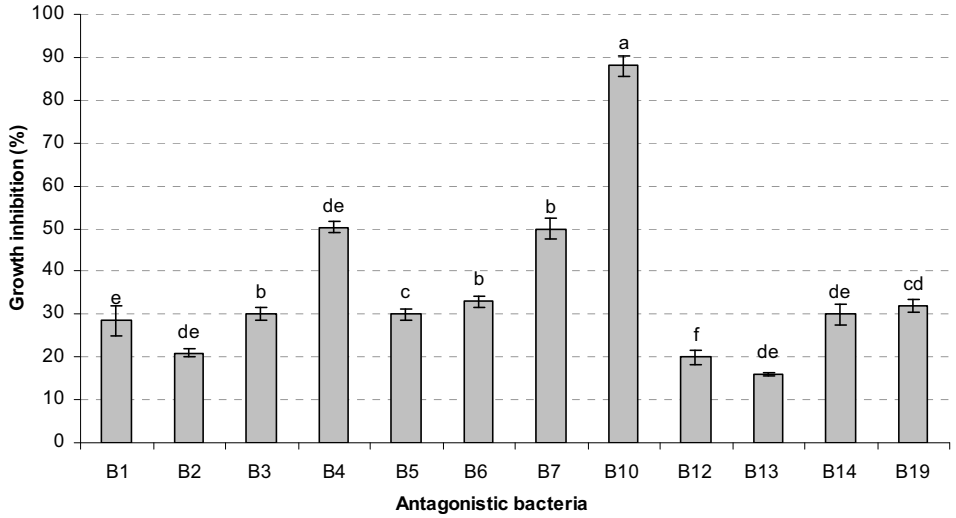

Fig. 1 *In vitro* effect of some antagonistic bacteria on the mycelial growth of *Pythium aphanidermatum* recorded after 3 days of incubation at 25°C. Bars with the same letters are not significantly different according to the Duncan's multiple range test (at $P \leq 0.05$).

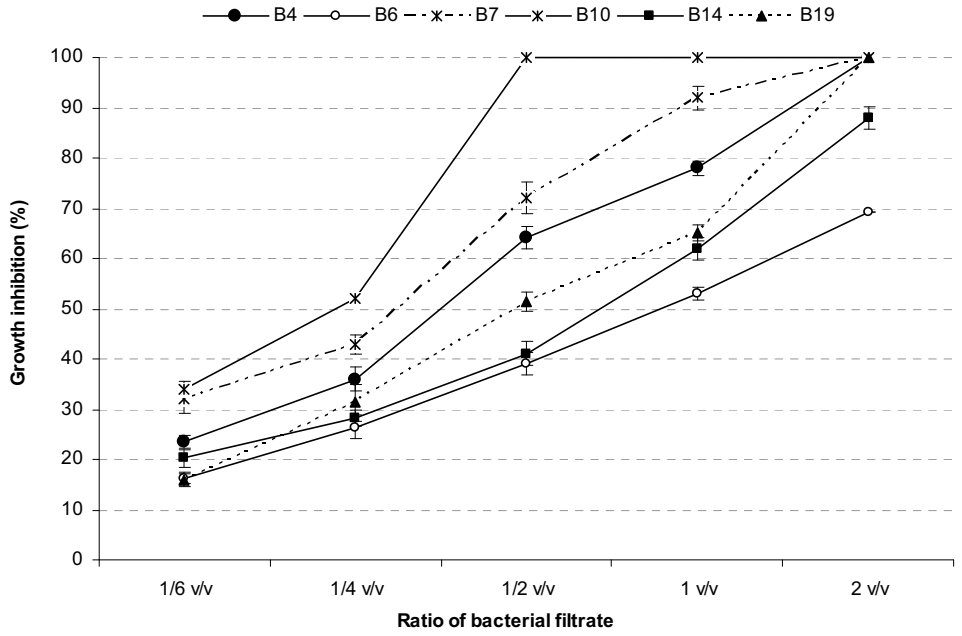

Fig. 2 Effect of antagonistic bacterial filtrates applied at different ratios on the mycelial growth of *Pythium aphanidermatum* recorded after 3 days of incubation at 25°C.

B_3, B_5, B_6, B_{12}, B_{14} and B_{19}), the recorded inhibition percentages varied between 20 and 33% as compared to the untreated control growth.

Effect of diffusible metabolites

When examined after an incubation period of 3 days, all the bacterial filtrates, added to PDA at 1/6, 1/2, 1/1, 2/1 (v/v) ratios, showed an important antifungal activity against *P. aphanidermatum* as compared to the untreated control (**Fig. 2**). The obtained results revealed important reductions in the pathogen colony diameters subsequent to the addition of bacterial culture filtrates to the culture medium. The recorded inhibition percentages increased proportionally with the increase of the culture filtrates volume. *P. fluorescens* (B_{10}) was shown to be the most efficient by exhibiting the highest antagonistic activity; pathogen growth was inhibited by 34 to 100% as compared to the untreated control with all tested culture filtrates ratios. Total inhibition of *P. aphanidermatum* growth was achieved with B_{10} isolate (*P. fluores-cens*) applied at the ratio of 1/2 (v/v) and also with B_4 (*A. xylosoxidans*), B_7 (*P. putida*) and B_{19} (*P. pseudoalcaligenes*) based treatments used at the strongest ratio i.e. 2/1 v/v (**Fig. 2**).

Evaluation of the ability of antagonistic bacteria to suppress the atypical pink rot of potato caused by *P. aphanidermatum*

According to the data from tuber experiments, the results obtained showed that all the treatments with antagonistic bacteria were significantly effective (P = 0.01) in controlling pink rot infection when tubers were treated 12 h before and after their inoculation for preventive and curative treatments, respectively (**Fig. 3**). A total rot was observed on the untreated and inoculated tubers whereas important reductions of decay severity were obtained with all biological treatments tested. When used as preventive treatments, the antagonists *P. fluorescens* (B_{10}), *Pseudomonas* spp. (B_{12}), *P. putida* (B_7), *Bu. cepacia* (B_1), and *A. xylosoxidans* (B_4) decreased rotting by 95, 89, 87, 82 and 80%, respectively as compared to the untreated control. The antagonist *P. fluorescens* was shown to be the most effective treatment when applied as curative or preventive treatment. However, except *Pseudomonas* spp., the other bacterial isolates reduced rot severity by 37 to 74% as compared to untreated control tubers.

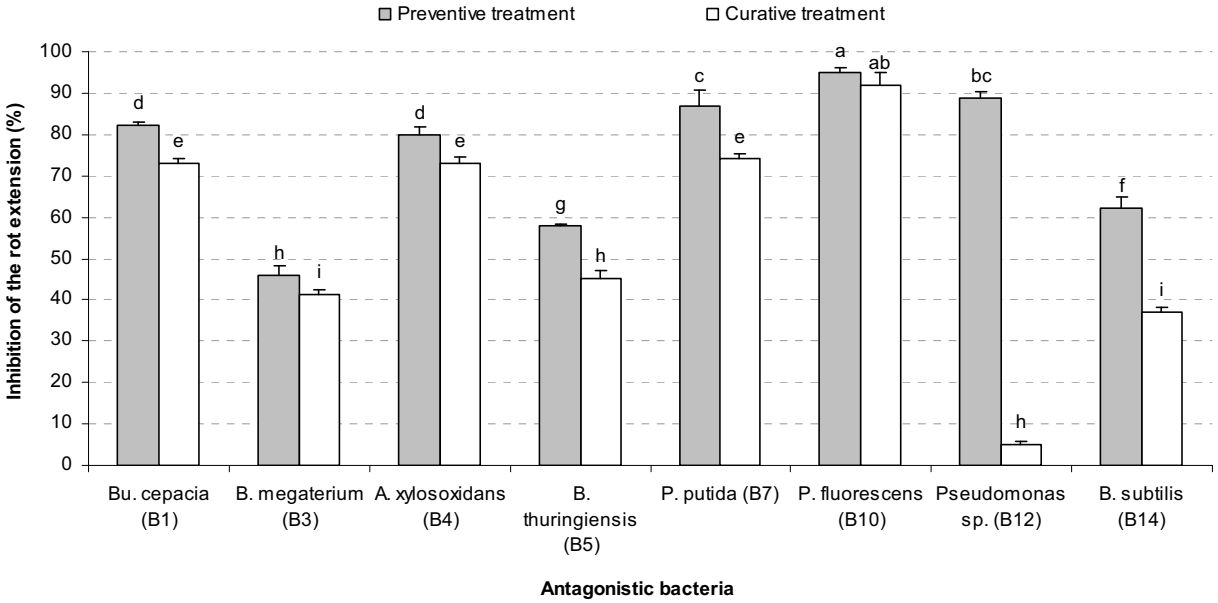

Fig. 3 Effect of autochthons antagonistic bacteria on the extension of the atypical pink rot of potato caused by *Pythium aphanidermatum* according to preventive and curative modes of treatment noted after 3 days of incubation at 25°C. Bars with the same letters are not significantly different according to the Duncan's multiple range test (at $P \leq 0.05$).

DISCUSSION

The use of biological control based on natural microorganisms offers a powerful alternative to synthetic chemical control of plant diseases. In fact, the abuse of chemical control agents, such as pesticides or fungicides, to cure or prevent plant diseases has often been reported to bring about a wide array of pernicious effects, particularly on plant, soil, environment and, ultimately, on human beings.

In view of this continuing search for new natural alternatives to chemical control agents, the present study was undertaken to investigate the potential use of indigenous bacterial isolates for the biological control of *P. aphanidermatum* the causal agent of atypical pink rot of potato in Tunisia.

Preliminary findings from biological control experiments carried out *in vitro* revealed that ten isolates, namely, *Bu. cepacia* (B_1), *B. megaterium* (B_3), *A. xylosoxidans* (B_4), *B. thuringiensis* (B_5), *Pseudomonas* sp. (B_6), *P. putida* (B_7), *P. fluorescens* (B_{10}), *Pseudomonas* sp. (B_{12}), *B. subtilis* (B_{14}), and *P. pseudoalcaligenes* (B_{19}) were effective in reducing *P. aphanidermatum* growth *in vitro.*

Further assays indicated the ability of the majority of antagonists to produce diffusible extracellular metabolites in the bacterial culture filtrates. Moreover, *P. fluorescens* was found to be the most efficient by exhibiting the highest inhibition.

The biological treatments of inoculated tubers by the bacterial filtrates were also efficient by reducing the rotting by more than 90% with *P. fluorescens* as compared to the untreated and inoculated tubers. In fact, some pseudomonads have been recognized as antagonists of plant fungal pathogens (O'Sullivan and O'Gara 1992). This is probably due to the abundance of this diverse group of bacteria and their obvious importance in the soils (Dowling and O'Gara 1994).

Rhizospheric *Pseudomonas* strains were reported to be effective for the control of a wide range of fungal and bacterial diseases (Kim *et al*. 2003). Bacteria of the genus *Pseudomonas* comprise a large group of the active biocontrol strains as a result of their general ability to produce a diverse array of potent antifungal metabolites. These include simple metabolites such as 2,4-diacetylphloroglucinol, phenazine-1-carboxylic acid and pyrrolnitrin [3-chloro-4-(2'-nitro-3'-chlorophenyl)-pyrrole], as well as the complex macrocyclic lactone, 2,3-de-epoxy-2,3-didehydra-rhizoxin. Pyrrolnitrin is active against *Rhizoctonia* spp., *Fusarium* spp., and other plant pathogenic fungi, and it has been used as a lead structure in the development of a new phenylpyrrole agricultural fungicide (Ligon *et al*. 2000). Several antibiotic-like substances have been identified, including bacteriocins and phenazine antibiotics (Hamdan *et al.* 1991). Moreover, *Pseudomonas* strains showed antifungal activity against *Verticillium dahliae* var. *longisporum in vitro* and were evaluated as potential biocontrol agents by Berg *et al*. (1998). Furthermore, *Pseudomonas* sp. controlled stalk rot of maize associated with *P. aphanidermatum* and *F. graminearum* at the seedling stage (Chen *et al*. 1999). Also, Janisiewicz and Roitman (1988) have also reported that blue mold and grey mold of apples and pears could be controlled by *Pseudomonas*.

Strains of *P. fluorescens* showed known biological control activity against certain soil-borne phytopathogenic fungi. In fact, Rajappan and Ramaraj (1999) evaluated the *in vitro* efficacy of *P. fluorescens* against the cauliflower wilt pathogen *F. moniliforme*. Fluorescent pseudomonad strains were also found to be effective against *Sclerotium rolfsii* (Patil *et al.* 1998) under greenhouse conditions in limiting groundnut and collar rot incidence. Thomashow *et al*. (1990) showed that the production of phenazine-1-carboxylic acid by *P. fluorescens* in the rhizosphere of wheat was correlated with take-all disease control. Moreover, antibiotics produced by *P. fluorescens* can also inhibit *P. ultimum* in the cotton spermosphere and rhizosphere (Howi and Suslow 1991). Some *Pseudomonas* species can also produce levels of hydrogen cyanide (HCN) that are toxic to certain pathogenic fungi (Dowling and O'Gara 1994). These characteristics make *Pseudomonas* species good candidates for use as seed inoculant and root dips for biological control of several soil-borne plant pathogens. Studies by Thomashow and Weller (1990) reviewed the importance of the antibiotic phenazine-1-carboxylic acid, produced by strain 2-29 of *P. fluorescens*, to suppress take-all disease of wheat caused by *Gaeumannomyces graminis* f. sp. *tritici*. Leaf application of *P. fluorescens* effectively conferred resistance against leaf pathogens (Hoffland *et al.* 1996) and has the potential to produce known secondary metabolites such as siderophore, HCN and protease that showed antagonistic activity against *Macrophomina phaseolina*, *Rhizoctonia solani*, *Phytophthora nicotianae* var. *parasitica*, *Pythium* sp. and *Fusarium* sp. (Hamdan *et al.* 1991).

In conclusion, the selected antagonists, especially *P. fluorescens*, hold a number of properties that might open

new opportunities for the biological control of the atypical pink rot disease. Accordingly, further studies are currently under way to further characterize and purify the antagonistic compounds produced by these antagonistic bacteria to experiment with their application in storage conditions and in the field. A biocontrol system using these strains may offer a good alternative to the currently employed chemical compounds, whose application entails a number of serious environmental and health hazards.

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REFERENCES

Berg G, Marten P, Bahl H (1998) Population dynamics of bacteria including antifungal species in the rhizosphere of oilseed rape during its life cycle. *Archives of Phytopathology and Plant Protection* **31**, 215-224

Callan NW, Mathre DE, Miller JB (1990) Bio-priming seed treatment for biological control of *Pythium ultimum* preemergence damping-off in *sh2* sweet corn. *Plant Disease* **74**, 368-372

Chen J, Gao HM, Lin RM, Ji MS, Gao ZG (1999) Infection mechanism and biocontrol of major corn fungal diseases in Northern China. *Report of Research Progress in Plant Protection and Plant Nutrition*, pp 78-84

Daami-Remadi M (1995) Inventaire, fréquence et agressivité des différentes espèces ou variétés de *Fusarium* rencontrées sur la pomme de terre ou dans son environnement en Tunisie. Mémoire de fin d'étude du cycle de spécialisation de l'INAT, Tunis, Tunisia, 115 pp (in French)

Daami-Remadi M (2001b) Activité antagoniste de *Trichoderma harzianum vis-à-vis* de *Pythium aphanidermatum* et de *Pythium ultimum* agents responsables de la pourriture aqueuse des tubercules de pomme de terre. *Annales de l'INRAT* **74**, 167-186 (in French)

Daami-Remadi M (2006) Etude des Fusarioses de la pomme de terre. PhD thesis, Ecole Supérieure d'Horticulture et d'Elevage de Chott-Mariem, Tunisia, 238 pp (in French)

Daami-remadi M, El Mahjoub M (2006) Présence en Tunisie d'isolats de *Fusarium sambucinum* résistants aux Benzimidazoles: Développement *in vitro* et agressivité sur tubercules de pomme de terre. *Biotechnologie, Agronomie, Société et Environnement* **10**, 7-16 (in French)

Dowling DN, O'Gara F (1994) Metabolites of *Pseudomonas* involved in the biocontrol of plant disease. *Trends in Biotechnology* **12**, 133-141

Hamdan H, Weller DM, Thomashow LS (1991) Relative importance of fluorescent siderophores and other factors in biological control of *Gaeumannomyces graminis* var. *tritici* by *Pseudomonads fluorescens* 2-79 and M4-80R. *Applied and Environmental Microbiology* **57**, 3270-3277

Howie WJ, Suslow TV (1991) Role of antibiotic biosynthesis in the inhibition of *Pythium ultimum* in cotton spermosphere and rhizosphere by *Pseudomonas fluorescens*. *Molecular Plant-Microbe Interactions* **4**, 393-399

Hultberg M, Alsanius B, Sundin P (2000) *In vivo* and *in vitro* interactions *Pseudomonas fluorescens* and *Pythium ultimum* in the suppression of damping-off in tomato seedlings. *Biological Control* **19**, 1-8

Janisiewicz WJ, Roitman J (1988) Biological control of blue mold and gray mold on apple and pear with *Pseudomonas cepacia*. *Phytopathology* **78**, 1697-1700

Kim HS, Park J, Choi SW, Choi KH, Lee GP, Ban SJ, Lee CH, Kim CS (2003) Isolation and characterization of *Bacillus* strains for biological control. *The Journal of Microbiology* **41**, 196-201

Ligon JM, Hill DS, Hammer PE, Torkewitz, NR, Hefmann DK, Kempfy HJ, Van Pée KH (2000) Natural products with antifungal activity from *Pseudomonas* biocontrol bacteria. *Pest Management Science* **56**, 688-695

Mulrooney RP (1982) Evaluation of ridomil for pink rot and leak control. *Fungicide and Nematicide Test* **37**, 78

O'Sullivan DJ, O'Gara F (1992) Traits of fluorescent *Pseudomonas* spp. Involved in suppression of plant root pathogens. *Microbiological Reviews* **56**, 662-676

Patil R, Jagadeesh K, Krishnaraj P, Kulkarni J (1998) Bacterization of groundnut with *Pseudomonas fluorescens* for the control of collar rot caused by *Sclerotium rolfsii* Sacc. Karnataka. *Journal of Agricultural Sciences* **11**, 423-425

Priou S, El Mahjoub M (1994) Incidence of bacterial and fungal diseases of potato in Tunisia. In: *Proceedings of the Third Triennial Conference of Potato African Association (APA)*, 9-13 May, 1994, Sousse, Tunisia, 120 pp (in English)

Priou S, El Mahjoub M (1999) Bacterial and fungal diseases in the major potato-growing areas of Tunisia. *EPPO/OEPP Bulletin* **29**, 167-171

Priou S, French E (1997) A simple baiting technique to detect and quantify *Pythium aphanidermatum* in soil. *Fitopatología* **32**, 187-193

Rajappan K, Ramarej B (1999) Evaluation of fungal and bacterial antagonists against *Fusarium moniliforme* causing wilt of cauliflower. *Annals of Plant Protection Sciences* **7**, 205-207

Roy S, Singh BP, Bhattacharyya SK, Shekhawat GS (1992) Distribution and control of pink rot of potato in Shimla Hills. *Journal of the Indian Potato Association* **19**, 45-49

Thomashow LS, Weller DM, Bonsall RF, Pierson LS (1990) Production of the antibiotic phenazine-1-carboxylic acid by fluorescent *Pseudomonas* species in the rhizosphere of wheat. *Applied and Environmental Microbiology* **56**, 908-912

Torres H, Martin C, Henfling J (1985) Chemical control of pink rot of potato (*Phytophthora erythroseptica* Pethyb.). *American Potato Journal* **62**, 335-361

Trapero-Casas A, Kaiser WJ, Ingram DM (1990) Control of Pythium seed rot and preemergence damping-off of chickpea in the U.S. Pacific Northwest and Spain. *Plant Disease* **74**, 563-569

Triki MA, Priou S, El Mahjoub M (2001a) Effects of soil solarisation on soilborne populations of *Pythium aphanidermatum* and *Fusarium solani* on the potato crop in Tunisia. *Potato Research* **44**, 271-279

Triki MA, Priou S, El Mahjoub M, Baudry A (2001b) Leak syndrome of potato in Tunisia caused by *Pythium aphanidermatum* and *Pythium ultimum*. *Potato Research* **44**, 221-231

Triki MA, Krid Hadj-Taieb S, Hammemi Mellouli I, Rhouma A, Gdoura R, Hassaïri A (2012) Identification and screening of bacterial isolates from Saharien weeds for *V. dahlia* control. *Journal of Plant Pathology* **94 (2)**, in press

Zinc RT (1995) The effect of metalaxyl on the incidence of pink rot. *American Potato Journal* **72**, 665-666

Weller DM (1988) Biological control of soilborne plant pathogens in the rhizosphere with bacteria. *Annual Review of Phytopathology* **26**, 397-407

Weller DM, Raaijmakers JM, McSpadden Gardener BB, Thomashow LS (2002) Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annual Review of Phytopathology* **40**, 309-348