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# Exploring rhizosphere and potato microbiome as potential antagonist to control blackleg and potato soft rot diseases in Morocco

Nisrine Sbai Idrissi*, Aicha Ouarzane, Latifa Elouazni, Aziz Hmyene, Said Elantri and Abdessamad Amine

## Abstract

**Background:** Blackleg and tuber soft rot are among the most important potato diseases caused by the bacteria belonging to the genera *Pectobacterium*. This pathogen causes significant economic losses each year. The antagonistic activity of different bacterial cultures against this pathogen was studied.

**Results:** Six hundred eight bacterial cultures isolated from potato tubers and rhizosphere soils procured from different locations across Morocco were tested for their antagonistic activity against *Pectobacterium carotovorum*. Forty isolates, all originating from tubers, showed positive antagonistic activity during preliminary screening. Among the 40 isolates, 10 were found to have a symptom suppression superior to 90%. Of the 10 isolates, 9 showed clear zone in the agar medium (in vitro test), with differences between antagonist's inhibition diameter. For the in vivo test, 8 isolates induced total suppression of soft rot on potato slices (in vivo test). The other 2 biocontrol strains (Amo-23 and Atd-2) were capable to minimize soft rot symptoms of up to 94.4 and 96.2%, respectively. Among the selected strains for in planta experiment, 6 strains (namely Ame-4, Atd-2, Atd-4, Ag-216, Al-51, and Ama-501) showed total reduction of disease symptoms. Biochemical and molecular tests identified 8 strains of *Bacillus* sp. and 2 strains of *Pseudomonas* sp.

**Conclusions:** The results of the in vivo and the greenhouse experiments indicated that the selected isolates had a greatly significant effectiveness for suppressing blackleg and soft rot symptoms. The selected isolates could, therefore, be used as a biocontrol agent against blackleg and soft rot of potato.

**Keywords:** Potato, Soft rot, Blackleg, *Pectobacterium carotovorum*, Biocontrol agent, Morocco

## Background

Blackleg and tuber soft rot potato diseases are caused by a bacteria belonging to the genera *Pectobacterium* (previously named pectolytic *Erwinia* spp.). This pathogen is one of the most important bacterial pathogens of potato. It causes significant economic losses each year and becomes an ongoing problem in the global potato industry worldwide (Dees et al. 2017). Tuber contamination occurs in both the field and after harvest (De Boer 2002). Soft rot and blackleg can develop, respectively in plants and tubers from contaminated seed tubers, which were reported to be the major source of dissemination of the bacteria (Pérombelon 2002). Sbai Idrissi et al. (2017) showed that when non-certified seeds are used, the percentage of soft rot and blackleg can reach 100% in the field. These authors reported that the bacteria are present in the field even when certified seeds are used.

The control of blackleg and tuber soft rot of potato currently depends on chemicals (Azaiez et al. 2018), preventive measures and the combination of cultural methods (Abo-Elyousr et al. 2010). Chemical methods

* Correspondence: svtnisrine@gmail.com
Laboratoire de Biochimie Environnement et Agroalimentaire, Faculté des Sciences et Techniques de Mohammedia, Mohammedia, Morocco

explored have shown limited success to prevent the disease, because the pathogens are frequently protected within the inner parts of the plants such as the lenticels and the vascular system (Czajkowski et al. 2011). A latent infection and the rapid proliferation of the pathogen have been well established when environmental conditions, including free water, oxygen availability, and temperature, become favorable (Hélias et al. 2000; Czajkowski et al. 2010).

In Morocco, storage under low temperatures is one of the preventive methods utilized. However, only few growers can adopt it because of its high cost. Also, the traditional detection in plant material through visual examination for disease symptoms remains insufficient to ensure an effective control. Symptom-based identification may require long waiting periods as the incubation period of the disease is strongly dependent on the environmental conditions. Diagnostic difficulties and the possible co-infection of a single host by several subspecies can also significantly influence the symptom identification (Pérombelon 2002). The limited success of physical and chemical methods, and the preference of the grower and consumer for non-chemically treated fruits and vegetables, has led to increase interest for the development of alternative strategies to control the phytopathogenic bacteria. Use of biological control has been and is still being attempted. In many cases, the biological control, as alternative method respecting the environment and not of high cost, was implemented with a variable degree of success (Aliye et al. 2008; Sharma et al. 2009; Nguyen et al. 2018).

The objectives of this study were (i) to isolate antagonistic bacteria to soft rot and blackleg *P. carotovorum*, (ii) to screen the effective microbial antagonists which can coexist with pathogens, from natural habitat, and (iii) to determine the potential of antagonistic bacteria, using in vitro and in vivo tests.

## Methods

### Bacterial isolates and inocula

Six hundred and eight bacterial isolates were collected and isolated from samples of tubers and rhizosphere soils of infested and non-infested potato fields by soft rot pathogens from different locations across Morocco. The tubers were rinsed in 100 ml sterile distilled water (SDW) and the suspension was diluted from $10^{-1}$ to $10^{-6}$. 0.1 ml of each dilution was streaked onto LPG agar medium (yeast extract 0.5%, pepton 0.5%, glucose 1%, agar 1.5%). For the soil samples, one gram (1 g) of each was taken to an Erlenmeyer's and 100 ml of SDW was added to it. Flasks were shacked at 130 rpm for 10 min (Swadling and Jeffries 1996). Serial dilutions of $10^{-1}$ to $10^{-6}$ in SDW were done and 0.1 ml of each dilution was plated to the surface of Petri dishes containing the same media described above. The Petri dishes were kept at 27 °C. After 48 h, individualized colonies, morphologically different, were selected as candidate antagonists.

For bacterial inoculums preparation, a sample of each colony was streaked onto LPG agar medium. After 48 h of incubation at 27 °C, each isolate was transferred into Erlenmeyer flasks containing SDW and adjusted to the desired concentration using the spectrophotometer. The pathogen *P. carotovorum* (*Pc*) B1158$^{T}$, obtained from the CNRST of Morocco, was used as the reference strain.

### Screening for potential biocontrol agent

Antagonistic effect of all candidate antagonists of *Pc* B1158$^{T}$ was studied by using potato slices. All treatments in this study were repeated 5 times.

#### *Sample preparation*

Potato tubers were surface sterilized by immersing the tubers in 3% NaOCl solution for 1 min and washed 3 times in SDW. Slices (1 cm thickness) were then cut and placed on moist sterile paper in plastic bowl.

#### *Bioassay calibration*

Absorbance of the candidate antagonistic suspensions was measured spectrophotometrically at 580 nm ($10^{8}$ CFU/ml). Firstly, each potato slice was inoculated by 1 ml of each candidate antagonist suspension. Then, the treated slices were inoculated with 100 μl of the pathogen suspension ($10^{7}$ CFU/ml). For the positive control, the potato slices were inoculated only with *Pc* B1158$^{T}$ reference strain. SDW was used as negative control. After 48 h of incubation at 27 °C, the percentage of inhibition was calculated by measuring the weight of the soft rot.

### In vitro antagonistic activity assay

The antibiosis activity was tested by agar diffusion technique (Guang-Hai et al. 2008). Suspensions (10 ml) of plant pathogenic bacterium *Pc* B1158$^{T}$ (around $10^{9}$ CFU/ml) were mixed with LPG agar medium (100 ml) prior to pouring into plates. After solidification, 2 μl suspension of each antagonistic isolate (around $10^{9}$ CFU/ml) was placed at the center of the agar surface and incubated at 27 °C for 48 h. Antibacterial activity was measured and defined by the appearance of a zone of inhibition. The diameter of the inhibition zone of selected isolates was calculated. All treatments in this study were done as three replicates. SDW was used as negative.

### In vivo antagonistic activity on potato plants

To evaluate the antagonistic activity in planta for the 10 selected isolates, a greenhouse study was conducted: seeds were first sterilized by immersing them in 3%

NaOCl solution for 1 min and washed three times in SDW. Then, the seeds were dipped for 15 min into each antagonist suspension of the 10 isolates (at $10^8$ CFU/ml). After that, the seeds were planted into pots (20 cm in diameter) containing around 3 kg of natural field soils as 5 replicates. Each seed was sprayed by 100 μl of the pathogen suspension (at $10^7$ CFU/ml). SDW and the reference strain of *Pc* B1158$^T$ were used as negative and positive control, respectively. Two-day old bacterial cultures were used for this assay. The humidity in the climate room was maintained around 70% RH and temperature was adjusted to 27 °C. The antagonistic activity was evaluated using a Disease Symptom Score (DSS) according to an arbitrary 0–4 scale (Aysan et al. 2003) where 0 = no symptom, 1 = 0.1–1.0 cm systemic infection on vessels, 2 = 1.1–3.0 cm systemic infection on vessels, 3 = 3.1–4 cm systemic infection on vessels or soft rot on stem, 4 = inhibition of the germination and the growth.

### Statistical analysis

Mann-Whitney *U* test was used to compare the results obtained by different antagonists with those obtained by positive and negative control.

### Identification of the antagonistic strains

#### *Biochemical characterization*

Biochemical identification of the 10 isolates was performed using the miniaturized multi-test identification system API 20E (Biomerieux). The 20 biochemical test reactions of the API 20E strips were inoculated by the 10 bacterial suspensions. All strips were incubated at 27 °C for 24 h. In addition, different biochemical test reactions were done for all bacterial suspensions: Gram; Catalase; King B; 6% and 3% NaCl; 39°C, 37 °C, and 27 °C; pH 5, 6, 7, and 8.

#### *Molecular characterization*

Genomic DNA from each of the 10 isolates was extracted from overnight culture, using a phenol-chloroform purification method, followed by an ethanol precipitation as described by Wilson (1987). Quantity and quality control of the DNA were measured using a NanoDrop ND 8000 and agarose gel electrophoresis at 1.0%.

The selected strains were identified by molecular feature. The amplification of the housekeeping genes, 16s rRNA, was done using the universal primers 1492r (5′ GGYTACCTTGTTACGACTT3′) and 27f (5′ AGAGTTTGATCMTGGCTCAG3′). Both primers are specific for the domain bacteria. Each reaction tube contained 25 μl of 1× PCR buffer (Bioline), a 2.5 U of *Taq* polymerase (Invitrogen) and each primer at a concentration of 0.5 μM and 1 μl of genomic DNA. The following parameters were used in all cycle sequencing reactions: initial denaturation step at 95 °C for 4 min, 35 cycles of denaturation at 95 °C for 30 s, 54 °C for 30 s, followed by an extension at 72 °C for 1 min and 30 s. Amplified DNA was examined by horizontal electrophoresis in 1% agarose with 5 μl aliquots of PCR products. Gel was stained with Ethidium bromide (0.5 μg/ml). The gels were viewed and photographed under UV Transilluminator gel doc Bio-Rad. All samples were purified and sequenced at the CNRST of Morocco. Finally, sequences were compared by sequences deposited in the GenBank nucleotide sequence database by using the Basic Local Alignment Search Tool (BLAST) program and were subsequently aligned with 16S rRNA reference sequences in the ARB package.

## Results

### Screening for potential biocontrol agent

The effect of antagonistic bacteria on the symptoms suppression of soft rot of potato tubers due to *Pc* B1158$^T$ was tested. During preliminary screening of the 608 bacterial isolates, 40 isolates, all originating from tubers, were found to inhibit growth of the pathogen on potato slices. In comparison to the control (slices inoculated with *Pc* B1158$^T$ only), the symptoms of soft rot produced by *Pc* were significantly retarded on potato slices (Fig. 1). The symptoms suppression of the tested isolates on potato slices were superior to 50%. Among the 40 isolates, 10 were found to have a symptom suppression superior to 90% (Ame-4, Amo-22, Amo-23, Atd-2, Atd-4, Ag-216, Ak-3, Ab-3, Al-51, and Ama-501). The isolates with symptom suppression inferior to 90% were discarded and the 10 selected ones were used for the following tests.

### In vitro study

Of the 10 selected isolates used in this study, 9 showed a clear effect against studied bacterium. The antagonistic activity was demonstrated by the appearance of clear zone in the agar medium (Fig. 2), which corresponds to the inhibition of the reference strain *Pc* B1158$^T$. However, there were differences between the isolates (Fig. 3). The diameters of inhibition vary from one isolate to another; the maximum values were recorded for Ag-216 and Amo-23 with 5.6 and 5.5 mm, respectively, indicating a strong antagonistic activity against the pathogen. The minimal value was recorded for Al-51 with a 3 mm diameter. Al-51 showed a zone which was not completely clear and thus there was delayed-action of the growth. On the other hand, the isolate Ab-3 did not exhibit any effect against *Pc* strains.

### Suppression of soft rot development on potato slices

The selected isolates were tested in a potato slice assay for their ability to reduce or suppress tuber soft rot caused by *Pc* B1158$^T$. The antagonistic bacteria showed a significant inhibition of the growth of the soft rot of

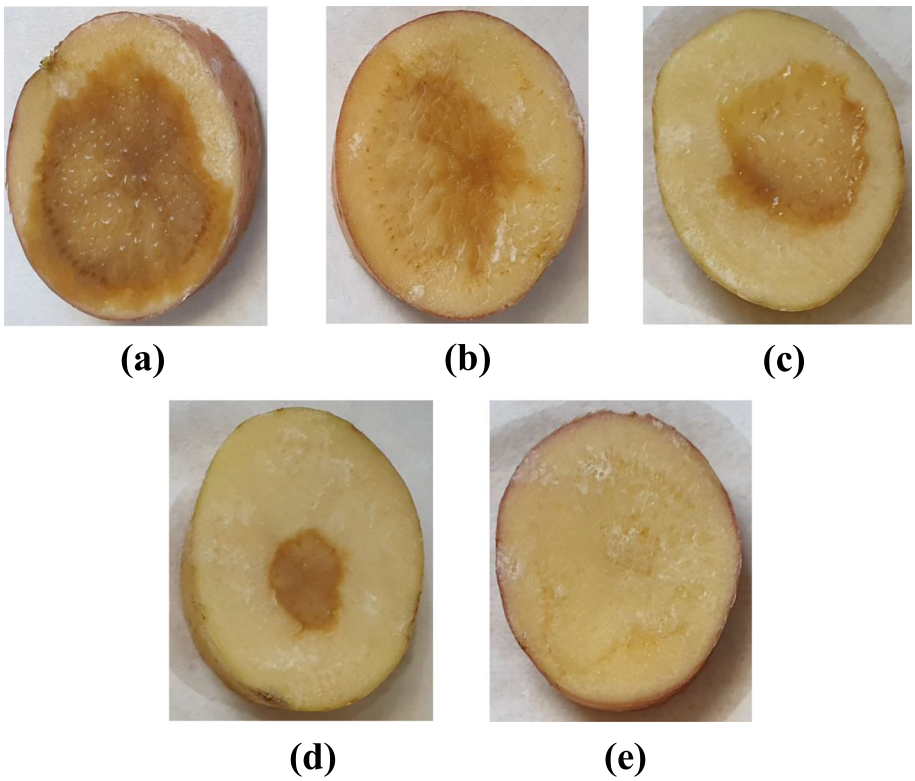


**Fig. 1** Effect of different antagonistic bacteria on suppression of soft rot development of potato. **a** Negative inhibition. **b**, **c**, and **d** are representatives of increased symptom suppression soft rot development. **e** Total inhibition of soft rot

the pathogen than the control. All the antagonists showed a restriction of the tissue maceration on potato slices to at least 91% of the control. Among the tested strains, eight induced total suppression of soft rot on potato slices. The other two strains (Amo-23 and Atd-2) were effective in reducing soft rot to up to 94.4 and 96.2%, respectively (Fig. 4).

### Greenhouse study (in planta)

A greenhouse study was conducted to determine the effect of the antagonistic activity against *Pc* B1158$^{T}$ in potato plants compared to the inoculated and untreated control plants (Table 1). The results showed that seed inoculation only with the pathogen significantly inhibited the germination, the growth and development of all the potato plant treated. For negative control, no disease symptoms were expressed and plants were able to grow. For seeds treated with both the antagonist and the pathogen, the experiment showed that 6 out of 10 isolates tested (namely Ame-4, Atd-2, Atd-4, Ag-216, Al-51, and Ama-501) completely inhibited the pathogen. These antagonists were able to provide total reductions

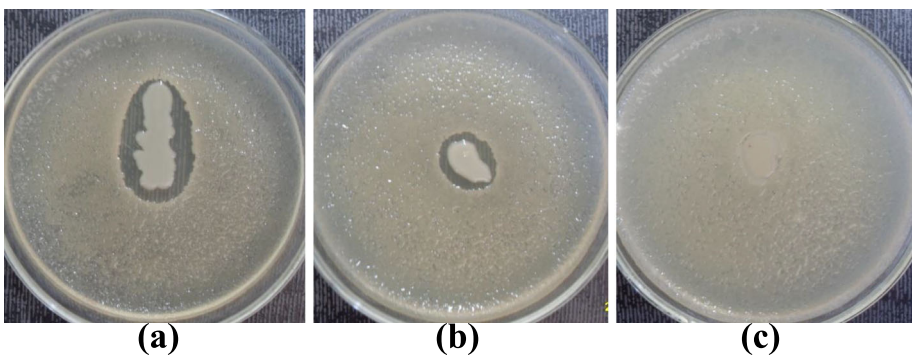


**Fig. 2** Antagonistic activity of isolates Amo-23 showing inhibition zones against soft rot bacterial strain *Pc* B1158$^{T}$. **a**, **b** are representatives of positive inhibition as shown by the encircled inhibition zones, and **c** is presenting negative inhibition as demonstrated by the no-inhibition zone

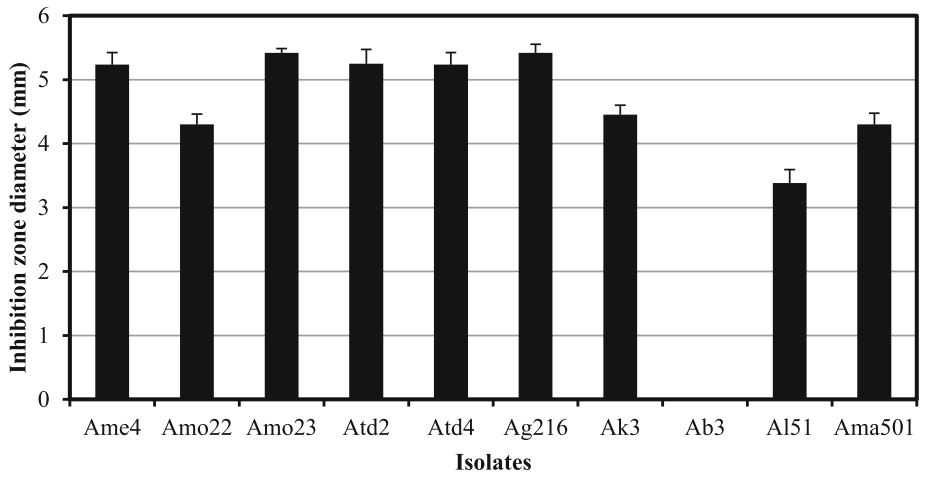


**Fig. 3** Inhibitory effect of selected antagonists on the growth of the strain *Pectobacterium carotovorum* B 1158$^T$

of disease symptoms. The disease symptoms were lowered by 70, 85, 90 and 95% using Amo-22, Ab-3, Amo-23, and Ak-3 isolates, respectively. In this case, the four antagonists were able to provide significant reductions of disease symptoms. However, symptoms of wilting appeared lately. To compare the antagonistic effect of the four strains (Amo-22, Ab-3, Amo-23, and Ak-3) with the positive and negative control, the Mann-Withney *U* test was used. Statistical analysis confirmed the antagonistic effect of the selected strains. The first test revealed highly significant differences ($p = 0.005$ for Amo-22, Ab-3, and Amo-23 and $p = 0.004$ for Ak-3) between the selected strains and the positive control. This test demonstrated that obtained isolates had an inhibitory effect on soft rot. The comparison to the negative control showed non-significant difference ($p > 0.05$) for the four antagonists.

### Identification of the antagonistic strains

The phenotypic, physiological, and biochemical characteristics of the selected antagonist isolates are listed in Table 2. All the isolates were Gram-positive and showed no fluorescence on King's medium B, with the sole exception of the isolate Atd-2, which was Gram negative and positive on King B. The biochemical properties such as catalase and gelatine production were positive for all the isolates. All the isolates showed a good growth at 39 °C and were negative for urease and $H_2S$ production. Among the 10 isolates, 8 were unable to tolerate NaCl at 6%. Only Ame-4 and Amo-23 were able to grow at NaCl

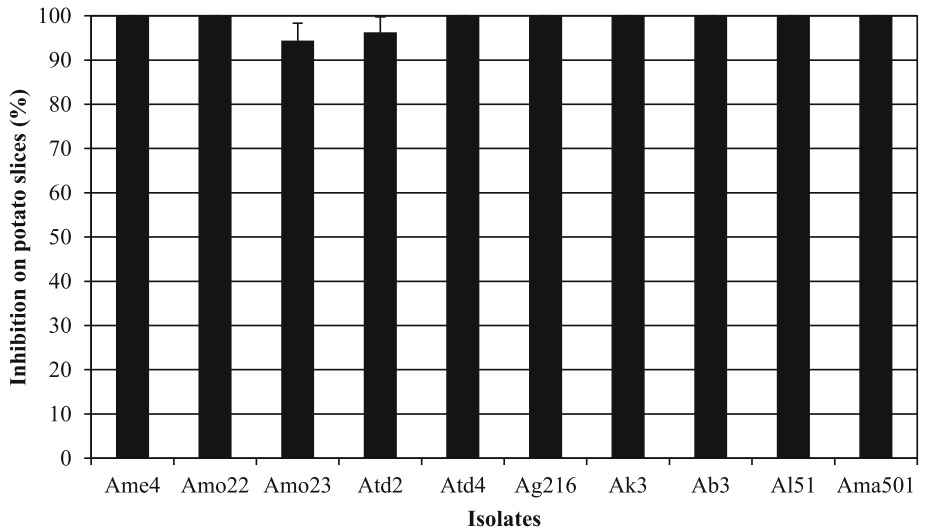


**Fig. 4** Biocontrol activity of antagonist bacteria against soft rot caused by *Pectobacterium carotovorum* B1158$^T$ on potato tuber slices. Suppression (%) = ((weight (g) of tissue soft rotted for the positive control–weight (g) of the tissue rotted)/ weight (g) of tissue soft rotted for the positive control) × 100

**Table 1** Variation in disease symptoms score (DSS) of inoculated potato plants

| Isolates | Average DSS[a] per isolate tested | Disease symptom decrease (%) |
|---|---|---|
| Ame-4 | 0 | 100 |
| Amo-22 | 1,2 | 70 |
| Amo-23 | 0,4 | 90 |
| Atd-2 | 0 | 100 |
| Atd-4 | 0 | 100 |
| Ag-216 | 0 | 100 |
| Ak-3 | 0,2 | 95 |
| Ab-3 | 0,6 | 85 |
| Al-51 | 0 | 100 |
| Ama-501 | 0 | 100 |
| Positive control | 4 | 0 |
| Negative control | 0 | 100 |

[a]Disease Symptom Score (DSS) estimated using an arbitrary 0–4 scale where *0* = no symptom, *1* = 0.1–1.0 cm systemic infection on vessels, *2* = 1.1–3.0 cm systemic infection on vessels, *3* = 3.1–4 cm systemic infection on vessels or soft rot on stem, *4* = inhibition of the germination and the growth. The visible symptoms first appeared on positive control plants

6%. The conventional characterization showed that 8 out of 10 antagonistic isolates (namely Ame-4, Amo-23, Amo-22, Atd-4, Ag-216, Ak-3, A1-51, and Ama-501) were *Bacillus* sp. while Atd-2 and Ab-3 were identified as *Pseudomonas* sp.

The results of the molecular identification of the 10 isolates, performed by using the method of 16S rRNA gene sequencing, are shown in (Table 3 and Fig. 5). The A260/A280 and A260/230 ratios were indicators for level of protein and organic contamination of DNA. DNA concentration measured by NanoDrop 8000 revealed an A260/A280 ratio between 1.88 and 2.19 and an A260/230 ratio between 1.08 and 2.43. These results showed a high purity of DNA extracted. After the sequencing of bacterial DNA extracted, the DNA sequences were analyzed with BLAST program. This test confirmed the results obtained by biochemical identification (Table 4). Eight antagonistic strains were identified as *Bacillus* sp. (namely Ame-4, Amo-23, Amo-22, Atd-4, Ag-216, Ak-3, A1-51, and Ama-501) and 2 as *Pseudomonas* sp. (Atd-2 and Ab-3).

## Discussion

Strains of *Pectobacterium* are the most important bacterial agent responsible of soft rot and blackleg diseases in potato tubers and plants, respectively. The biological control to protect plant growth against pathogens remains an active and a promising strategy among the preventive methods used, especially chemical and physical ones. Biocontrol is, hence, important in developing countries like Morocco where only few growers can utilize methods of high cost such as the storage under low temperatures. To this end, several organisms isolated from samples of tubers and rhizosphere soils of infested and non-infested potato fields by soft rot pathogens were selected for an eventual biological control of

**Table 2** Phenotypic, physiological, and biochemical characterization of the antagonists

| Parameter | Value by antagonist | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Ame4 | Amo22 | Amo23 | Atd2 | Atd4 | Ag216 | Ak3 | Ab3 | Al51 | Ama501 |
| Gram | + | + | + | − | + | + | + | - | + | + |
| Fluorescence on King B | − | − | − | + | − | − | − | − | − | − |
| ONPG | + | + | − | + | + | - | + | − | − | − |
| Catalase | + | + | + | + | + | + | + | + | + | + |
| Gelatin | + | + | + | + | + | + | + | + | + | + |
| Urease | − | − | − | − | − | − | − | − | − | − |
| $H_2S$ | − | − | − | − | − | − | − | − | − | − |
| Cell form | Bacill | Bacill | Bacill | Bacill | Bacill | Bacill | Bacill | Bacill | Bacill | Bacill |
| Tolerance to NaCl 6% | + | − | + | − | − | − | − | − | − | − |
| Growth at 39 °C | + | + | + | + | + | + | + | + | + | + |

**Table 3** Quantification of DNA extracted from the 10 antagonist strains

| Antagonistic strains | DNA concentration ng/μl | 260/280 ratio[a] | 260/230 ratio[a] |
|---|---|---|---|
| Ame-4 | 609.5 | 1.91 | 1.43 |
| Amo-23 | 416.9 | 2.19 | 2.42 |
| Amo-22 | 259.9 | 1.96 | 1.49 |
| Atd-4 | 332.3 | 2 | 1.59 |
| Ag-216 | 340.6 | 1.88 | 1.08 |
| Ak-3 | 978.7 | 2.03 | 1.56 |
| A1-51 | 354.6 | 1.92 | 1.33 |
| Ama-501 | 877.9 | 2.16 | 2.34 |
| Atd-2 | 1694 | 1.92 | 1.32 |
| Ab-3 | 1198 | 2.18 | 2.43 |

[a]Concentrations 260/280 and 260/230 ratios were obtained using NanoDrop 8000

potato diseases. The preliminary screening for potential agents allowed us to retain 10 isolates of the 608 tested isolates with a symptom suppression superior to 90%. The results indicated that these isolates could produce antibacterial substances targeting the growth of *Pectobacterium* pathogens. The screening of potential biocontrol agent was applied either in vitro or in vivo. Most of the published biocontrol assays aiming the screening of antagonistic microorganisms were directed firstly to in vitro experiments (El-Sayed et al. 2014; Des Essarts et al. 2016; Lin et al. 2018). However, it was noticed under the present experimental conditions, that some of the isolates could exhibit antagonistic activity in vivo but not in vitro. The use of biological control for the management of pathogens indicated in previous research that multiple mechanisms are more likely involved in the inhibition of plant pathogens: nutrient competition, production of antibiotics, degradative enzymes, and nitrous oxide (Mahmoudi et al. 2011) as well as competition for space and induction of resistance (Sharma et al. 2009). The pathogen and disease suppression were, in other research, associated with changes in the soil microbial communities (Smolinska 2000; Cohen et al. 2005). Some of these mechanisms, by which antagonists control the growth of postharvest diseases, could probably not appear in vitro. Hence, in this study we first tested the antagonistic activity of obtained isolates in vivo.

The results of the in vitro experiments indicated that 9 out of the 10 isolates screened exhibited antagonistic

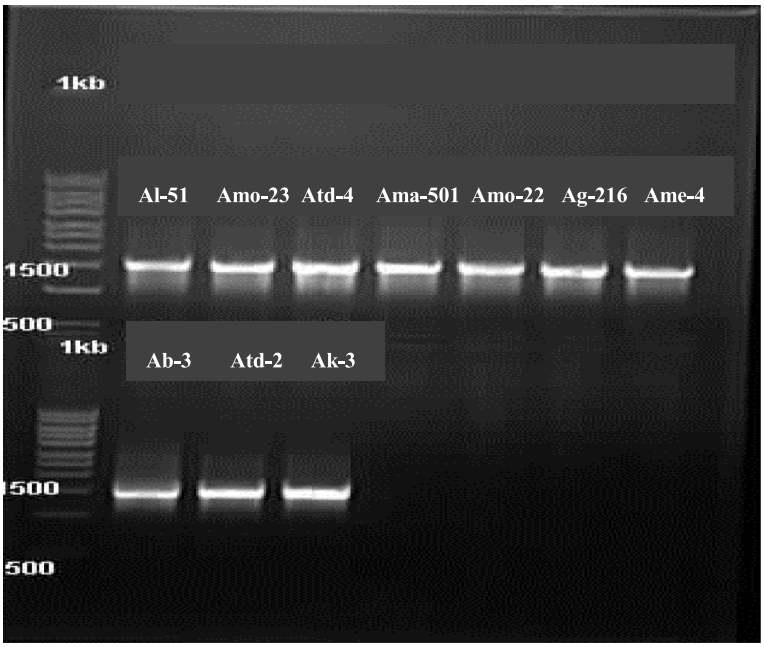


**Fig. 5** Agarose gel showing the quality of DNA extracted from the 10 antagonist strains

**Table 4** Comparison of the sequences obtained from the amplifiates of the RNA16S gene extracted from the antagonist strains using BLAST

| 27F-AGAGTTTGATCCTGGCTCAG | | | 1492r-GGTTACCTTGTTACGACTT | | |
|---|---|---|---|---|---|
| **Isolates** | **Descriptions** | **Nucleotide start position** | **Nucleotide end position** | **Bp length** | **BLAST resulted** |
| 1 | A2FD1-A9 | NP | NP | 491 | *Bacillus* sp. |
| | A2PDF-A11 | | | 504 | *Bacillus* sp. |
| | A2RP2-A10 | | | 514 | *Bacillus* sp. |
| 2 | A5FD1-B9 | 21 | 501 | 481 | *Pseudomonas* sp. |
| | A5FD1-B11 | 20 | 532 | 513 | *Pseudomonas* sp. |
| | A5RP2_B10 | 189 | 601 | 413 | *Pseudomonas* sp. |
| 3 | A8FD1_C9 | 50 | 452 | 403 | *Bacillus* sp. |
| | A8PDF_C11 | 40 | 372 | 330 | *Bacillus* sp. |
| | A8RP2_C10 | 16 | 551 | 536 | *Bacillus* sp. |
| 4 | A23FD1_D9 | 30 | 210 | 181 | *Bacillus* sp. |
| | A23PDF_D11 | 20 | 551 | 532 | *Bacillus* sp. |
| | A23RP2_D10 (1st part) | 19 | 231 | 213 | *Bacillus* sp. |
| | A23RP2_D10 (2nd part) | 280 | 480 | 202 | *Bacillus* sp. |
| 5 | A51FD1_E9 | 21 | 602 | 582 | *Bacillus* sp. |
| | A51PDF_E11 | 50 | 651 | 602 | *Bacillus* sp. |
| | A51-RP2_E10 | 55 | 572 | 602 | *Bacillus* sp. |
| 6 | A55-FD1_F9 | 17 | 320 | 306 | *Bacillus* sp. |
| | A55-FD1_F9 | 350 | 531 | 182 | *Bacillus* sp. |
| | A55-PDF-F11 | 70 | 600 | 533 | *Bacillus* sp. |
| | A55-RP2-F11 | 0 | 0 | 0 | - |
| 7 | A402FD1_G9 | 63 | 601 | 539 | *Bacillus* sp. |
| | A402PDF_G11 | 20 | 151 | 132 | *Bacillus* sp. |
| | A402RP2_G10 | 43 | 551 | 509 | *Bacillus* sp. |
| 8 | AB71FD1_H9 | 30 | 608 | 579 | *Bacillus* sp. |
| | AB71PDF_H11 (1st part) | 40 | 264 | 225 | *Bacillus* sp. |
| | AB71PDF_H11 (2nd part) | 344 | 544 | 202 | *Bacillus* sp. |
| | AB71RP2_H10 | 50 | 651 | 602 | *Bacillus* sp. |
| 9 | K44FD1_A12 | 180 | 382 | 203 | *Bacillus* sp. |
| | K44PDF_E12 | 40 | 603 | 564 | *Bacillus* sp. |
| 10 | R2FD1_B12 | 60 | 572 | 513 | *Pseudomonas* sp. |
| | R2PDF_F12 | 49 | 600 | 552 | *Pseudomonas* sp. |
| | R2RP2_D12 | 70 | 572 | 503 | *Pseudomonas* sp. |

effect on *Pc* B1158$^{T}$ strains by the appearance of clear zone of inhibition. The isolates Amo-23 and Ag-216 exhibited particular inhibition. However, only one isolate (Ab-3) did not exhibit any effect against *Pc* strains. These results are in agreement with those revealed by Salem and Abd El-Shafea (2018) who reported that, under in vitro experiments, the bioagents, *Bacillus subtilis*, *Pseudomonas fluorescens*, *P. aeruginosa*, and *Streptomyces* spp. showed an antagonistic effect against soft rot disease in potato tubers caused by *Erwinia carotovora* subsp. *carotovora*. These biocontrol agents exhibited values of inhibition zones up to 40 mm. It was also showed in an early study that the soft rot bacterial pathogen of potato *P. carotovorum* subsp. *atroseptica* was inhibited by the isolates from the *P. fluorescens* under in vitro conditions (Cronin et al. 1997).

The biocontrol effects of the isolates were also investigated using in vivo and in planta assays: the percentage of inhibition was calculated by measuring the weight of the soft rot on potato slices for the in vivo experiments, and the symptom development in greenhouse experiment. The results of the investigations demonstrated

that all identified antagonists could significantly inhibit the growth of soft rot and blackleg bacteria in vivo and in planta. Statistical analysis confirmed the antagonistic effect of the strains used on potato seeds. The present findings corroborated the results of Czajkowski et al. (2012) who showed that antagonistic isolates like *Bacillus* and *Pseudomonas*, tested in potato slice assay, had the potential bio protection against bacterial diseases. Their selected isolates were able to reduce rotting of potato tuber tissues by at least 50%. Babana et al. (2011) confirmed that the soft rot disease in Malian potato tubers caused by *Bacillus pumilus* could biologically be controlled in potato slices by using the isolates of actinomycetes. Salem and Abd El-Shafea (2018) found, recently, that the severity disease was highly decreased under greenhouse conditions (in pots) by using various species (*B. subtilis*, *P. fluorescens*, *P. aeruginosa*, and *Streptomyces* spp.) against *E. carotovora* subsp. *carotovora* isolates. Similarly, Algeblawi and Adam (2013) reported that the bioagents *P. fluorescens*, *B. subtilis* and *B. thuringiensis* reduced soft rot disease in potato tubers caused by *E. carotovora* subsp. *carotovora* in pot experiment.

In the present study, biochemical and molecular identifications suggest that identified antagonists bacterial strains belong to *Pseudomonas* sp. (2 strains; Ab-3 and Atd-2) and *Bacillus* sp. (8 strains; Ame-4, Amo-23, Amo-22, Atd-4, Ag-216, Ak-3, A1-51 and Ama-501). Many researchers identified various strains belonging to *Bacillus* sp. and *Pseudomonas* sp. and exploited their antagonistic effect to control pathogenic bacteria in different plants. *Bacillus subtilis* strains were tested for the control of potato diseases caused by *Pectobacterium* spp., and results revealed reduced maceration symptoms *in planta* (Gerayeli et al. 2017). Cladera-Olivera et al. (2006) reported that a bacteriocin-like substance produced by *Bacillus licheniformis* P40 was bactericidal to *Pectobacterium carotovorum* subsp. *carotovorum*. This substance interacted with cell membrane lipids, provoking lysis of *P. carotovorum* subsp. *carotovorum* cells. *Bacillus* species have been used as a biocontrol agent against different pathogenic fungi (Mates et al. 2019) and bacteria (Azaiez et al. 2018; Durairaj et al. 2018).

## Conclusions

The findings of this study showed a significant potential of bacterial cultures isolated from potato tubers against the bacteria of *Pectobacterium*. The antagonistic activity of the tested isolates by the reduction of blackleg and soft rot symptoms was proved under in vitro, in vivo, and in planta conditions.

### Abbreviations

SDW: Sterile distilled water; LPG: Levure peptone glucose; CNRST: Centre National pour la Recherche Scientifique et Technique; *Pc*: *Pectobacterium carotovorum*; CFU: Colony-forming unit; RH: Relative humidity; DSS: Disease Symptom Score; API: Analytical profile index; DNA: Deoxyribonucleic acid; RNA: Ribonucleic acid; PCR: Polymerase chain reaction; Taq: Thermus aquaticus; UV: Ultraviolet; BLAST: Basic Local Alignment Search Tool

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### Authors' contributions

SIN was responsible for methodology, investigation, statistical analysis and manuscript writing. AA was responsible for methodology and revised the manuscript. All authors have read and approved the manuscript.

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