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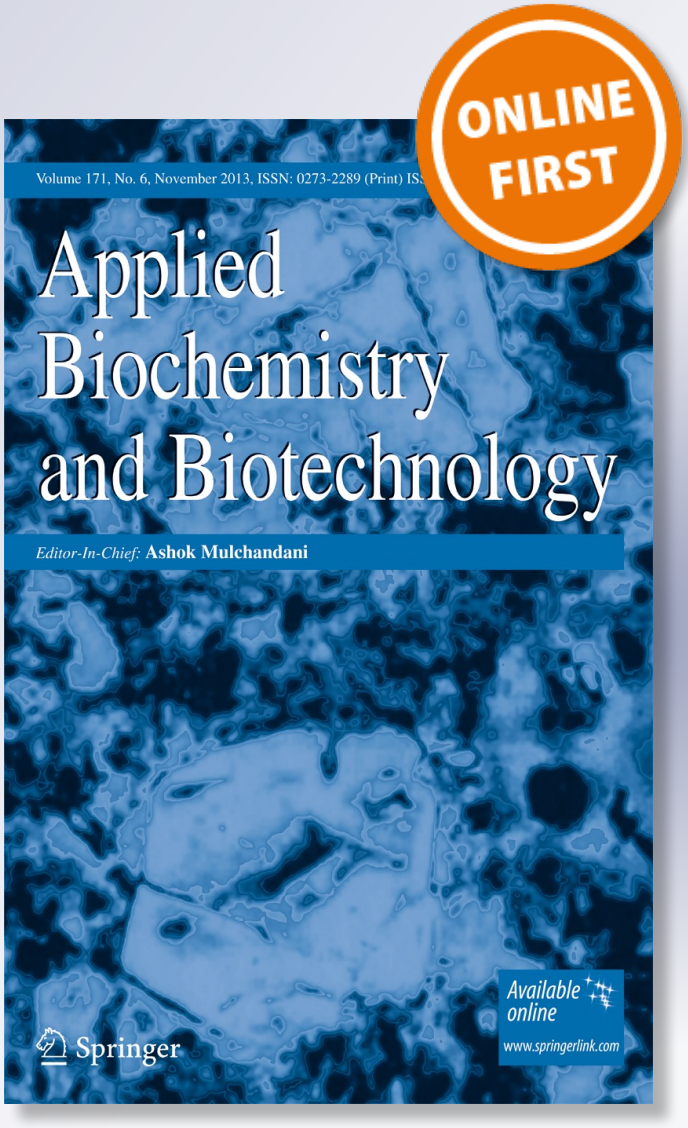


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# In Vitro and In Vivo Characterization of Plant Growth Promoting *Bacillus* Strains Isolated from Extreme Environments of Eastern Algeria

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**Abstract** This report is to our knowledge the first to study plant growth promotion and biocontrol characteristics of *Bacillus* isolates from extreme environments of Eastern Algeria. Seven isolates of 14 (50 %) were screened for their ability to inhibit growth of some phytopathogenic fungi on PDA and some roots exudates. The bacteria identification based on *16S r-RNA* and *gyrase-A* gene sequence analysis showed that 71 % of the screened isolates belonged to *Bacillus amyloliquefaciens* and the rest were closely related to *B. atrophaeus* and *B. mojavensis*. Most of them had high spore yields ($22\times10^8$–$27\times10^8$ spores/ml). They produced protease and cellulase cell wall-degrading enzymes while the chitinase activity was only observed in the *B. atrophaeus* (6SEL). A wide variety of lipopeptides homologous was detected by liquid chromatography–electrospray ionization–mass spectrometry analysis. Interestingly, some additional peaks with new masses were characterized, which may correspond to new fengycin classes. The isolates produced siderophores and indole-3- acetic acid phytohormone. The greenhouse experiment using a naturally infested soil with *Sclerotonia sclerotiorum* showed that the *B. atrophaeus* (6SEL) significantly increased the size of the chickpea plants and reduced the stem rot disease ($P<0.05$). These results suggest that these isolates may be used further as bio-inoculants to improve crop systems.

**Keywords** Extreme environments · *Bacillus* · Biocontrol · Plant growth promotion · Lipopeptides · IAA · Siderophores

A. Ait-Kaki (✉) · M. Ongena · P. Thonart
Wallon Center of Industrial Biology, University of Liege, B40-Sartilman, 4000 Liege, Belgium
e-mail: a.aitkaki@student.ulg.ac.be

A. Ait-Kaki · N. Kacem-Chaouche · M. Kara-Ali · L. Dehimat
Laboratory of Mycology, Biotechnology and Antimicrobial Activity, Department of Biochemistry-Microbiology, University Mentouri of Constantine 1, Ain Elbey, Constantine 25017, Algeria

K. Kahlat
College of Medicine, King Faisal University, PO. Box 400, Al-Ahsa 31982, Saudi Arabia

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## Introduction

Plant diseases are a major and chronic threat to food production and ecosystem stability worldwide; they are responsible for the loss of at least 10 % of global food production [1]. To increase crop yields and reduce damage from agricultural diseases, farmers use manufactured chemical fertilizers and pesticides. However, increasing application of such chemicals causes several negative impacts such as development of pathogen resistance to the applied agents, their nontarget environmental impacts, and many more problems including significant pollution of soils and groundwater reservoirs and accumulation of undesirable chemical residues in the food chain [2]. Consequently, there is an increasing demand from consumers and officials to reduce the use of chemical pesticides. In this context, the use of beneficial microorganisms could be an environmentally sound option to increase crop yields and reduce disease incidence [3].

Bacteria that exert beneficial effects on plant development include several genera such as *Acinetobacter*, *Agrobacterium*, *Arthrobacter*, *Azospirillum*, *Bacillus*, *Bradyrhizobium*, *Frankia*, *Pseudomonas*, *Rhizobium*, *Serratia*, *Thiobacillus* [4]. *Bacillus* sp. offer several advantages over other genera because of their capacity to produce spores in unfavorable environmental conditions. This phenomenon facilitates the conversion of spore suspension to powder formulations without the dramatic bacterial mortality observed with non-sporulating bacteria [5]. Numerous *Bacillus* isolates can improve plant health and productivity by three different ecological mechanisms; the antagonism of phytopathogens, the stimulation of plant host defenses, and the promotion of their nutrition and growth [6]. The antagonism developed by *Bacillus* strains is explained by the production of several antimicrobial compounds. Among the most studied antibiotics produced by *Bacillus* isolates are low molecular weight compounds that are synthesized by non-ribosomal mechanisms such as polyketides and lipopeptides. A major class of *Bacillus* peptide antibiotics are cyclic lipopeptides (cLPs) of the surfactin, iturin, and fengycin families that may vary in the type of amino acid residues, the nature of the peptide cyclization, and in the nature, length, and branching of the fatty acid chain [7]. In addition to their antagonism effect, the main natural functions of LPs from *Bacillus* strains described to date are their role in motility and attachment to surfaces [8] and their function as signal molecules for coordinated growth and differentiation in biofilm structures [9].

Several strains of *Bacillus* spp. have been demonstrated to stimulate plant defense responses; this phenomenon is termed "induced systemic resistance (ISR). Furthermore, lipopeptides and volatile compounds such as 2,3-butanediol [10] were defined as elicitors of ISR in *Bacillus* spp. Members of the genus *Bacillus* are among the most commonly reported plant growth promoting rhizobacteria (PGPR). In other PGPRs, termed "biofertilizers," the mechanisms that are involved in this process include nitrogen fixation, phosphate, and mineral solubilization, and the production of macromolecule degrading enzymes (amylases, proteases, and hemicellulases), phytohormones (indole-3 acetic acid, cytokinin, and gibberellins), volatile growth stimulants as ethylene, and 2,3-butanediol [11] and siderophores which chelate iron (Fe) and deprive the phytopathogenic fungi of it [12].

The extreme environments are interesting ecological resources for the isolation of stress resisting microorganisms with unusual phenotypic and genotypic characteristics [13, 14]. To our knowledge, the characterization of *Bacillus* isolates for their plant growth promotion and biocontrol capacities had never been carried out from such environments. The main objectives of the present study were to (1) isolate the predominant antifungal *Bacillus* sp. from the salt lake of Ain M'lila and the hot spring of Oued El Athmanya of Eastern Algeria, (2) identify them by analyzing *16S-rRNA* and *gyr-A* genes, (3) evaluate their in vitro capacity to produce

spores, antifungal compounds, i.e., cell wall-degrading enzymes and cLPs, indole-3 acetic acid phytohormone, and siderophores, (4) test the capacity of some of them to produce antifungal compounds in root exudates and to control chickpea plants growth in greenhouse conditions, in order to further use them as bio-inoculant strains.

## Materials and Methods

### Sampling Site and Isolation of Aerobic Endospore-Forming *Bacillus*

Samples of rhizosphere soil, soil, and water from the salt lake of Ain M'lila and the hot spring of Oued El Athmanya of Eastern Algeria were collected. One gram of soil samples was mixed with 9 ml of 0.9 % of sodium chloride (NaCl), and serial decimal dilutions were prepared, pasteurized (12 min, 80 °C) to eliminate non-sporulated bacterial forms. Then, the surviving spores corresponding to the *Bacillus* isolates were plated on a rich LB medium: composition in grams per liter: 10 tryptone, 5 yeast extract, 5 NaCl [15]. After 24 h of incubation at 30 °C, bacteria that had been grown were purified and stored by freezing at −80 °C.

### In Vitro Screening for Antagonism

The *Bacillus* isolates were tested on Petri dishes of potato dextrose agar (PDA) medium for their ability to inhibit the growth of various phytopathogenic fungi, such as *Botrytis cinerea*, *Aspergillus niger*, *Alternaria alternata*, *Fusarium oxysporum*, and *Cladosporium cucumerinium*. Five microliters of *Bacillus* suspension ($10^7$cellules/ml) were deposited on the edge of the plates, and mycelial plugs (5 mm) of each fungus were deposited in the center, approximately 3.5 cm from the bacterial colonies. Control plates not inoculated with bacteria were also prepared. Plates were incubated at room temperature for 2 to 6 days, depending on the tested fungus. Mycelia growth inhibition was expressed as the percentage of reduction of mycelium expansion compared with control plates without bacteria [16]. Means ± standard deviations were calculated from three replications used for each fungal strain.

### Determination of Spore Yields

The *Bacillus* isolates were grown in a liquid medium (named Opt medium) as described by Jacques et al. (1999) [17], at 30 °C and 180 rpm for 72 h. The bacterial suspensions obtained at the end of the culture were treated by heat chock at 80 °C for 12 min followed by immediate cooling to room temperature in cooled water. This heat treatment kills the vegetative form of bacteria and keeps only the resistance form (spores). The spreading of 100 μl of appropriate dilutions on LB medium allows us to measure colony forming unit per milliliter, which corresponds to spores per milliliter. All the experiments were performed as three replications.

### Species Identification of the *Bacillus* Isolates Based on 16S r-RNA and gyr-A Gene Sequences Analysis

The total DNA of the *Bacillus* isolates was extracted from liquid cultures with wizard Genomic DNA purification kit (Promega), according to the manufacturer's instructions. The primers used for the PCR amplification were the universal primers *16SP0* (GAA GAG TTT GAT CCT GGC TCAG) and *16SP6* (CTA CGG CTA CCT TGTTAC GA) for *16S r-RNA* gene [18] and *gyr-A.f* (CAG TCA GGA AAT GCG TAC GTC CTT) and *gyr-A.r* (CAA GGT AAT GCT

CCA GGC ATT GCT) for *gyr-A* gene [19]. The PCR products were purified using GFX PCR DNA and Gel Band purification kit. The same primers cited above were used for the sequencing reactions, and the obtained sequences were assembled and corrected using BioEdit program. These sequences were compared with other sequences in GenBank by using the BLASTN program. The obtained accession numbers from GenBank are resumed in Table 1.

## Determination of the Pattern of Lipopeptides

The *Bacillus* strains were grown in agitated flasks (180 rpm) containing the opt medium at 30 °C for 72 h. Cultures were centrifuged at 15,000 g for 20 min. The supernatant samples were loaded on C18 solid-phase extraction cartridges (900 mg, Alltech) and lipopeptides were desorbed with 100 % acetonitrile (ACN). The resulting samples were analyzed by reverse-phase HPLC coupled with single quad mass spectrometer (HPLC Waters Alliance 2695/diode array detector, coupled with Waters SQD mass analyzer) on a X-terra MS (Waters) 150*2.1 mm, 3.5 μm column as previously described by Nihorimbere et al. (2012) [20]. In this work, a single elution gradient allowing the simultaneous measurement of all three lipopeptide families was used. The water acidified with 0.1 % formic acid (A) and ACN acidified with 0.1 % formic acid (B) were used as a mobile phase. The flow rate was maintained at 0.5 mL $min^{-1}$ and the column temperature at 40 °C, with a gradient of 35 min (43–80 %, v/v ACN in 18 min; 100 %, v/v ACN for 9 min; and 43 %, v/v ACN in 8 min). Compounds were first identified on the basis of their retention times compared to purified standards and the amounts were calculated on the basis of the corresponding peak area (max plot). The identity of each homologue was confirmed on the basis of the masses detected in the SQD by setting electrospray ionization conditions in the MS as source temp., 130 °C; desolvation temp., 250 °C; nitrogen flow, 500 l/h; cone voltage, 70 V. The positive ion mode was used for analysis of all three families because a higher signal/background ration was obtained compared to negative ion recording.

**Table 1** Geographical identification and spore yields of *Bacillus* strains isolated from extreme environments of Eastern Algeria

| Sampled site (number of antagonistic *Bacillus* strains/ number of the total isolated *Bacillus* strains ) | Antagonistic *Bacillus* strains[a] (genbank accesion number) | Spore yields (×108 cfu/ml) |
|---|---|---|
| Rhizosphere of plant located near a salt lake (Ain M'lila, Algeria): 2/8 | (Rh2. A') *B. amyloliquefaciens 16S*-KC341736/*gyrA*-KC204920 | 22.7±0.6 |
| | (Rh2. F) *B. amyloliquefaciens 16S*-KC341737/*gyrA*-KC-204921 | 24.7±0.6 |
| Salted water tables (Ain M'lila, Algeria): 1/1 | (ET) *B. amyloliquefaciens 16S*-KC341738/*gyrA*-KC-204922 | 24.3±0.6 |
| Soil located 1 m from the hot spring (Oued El Athmanya, Algeria): 3/3 | (SEL) *B. amyloliquefaciens 16S*-KC341739/*gyrA*-KC-204923 | 25.0±2.0 |
| | (6SEL) *B. atrophaeus 16S*-KC341740/*gyrA*-KC-204925 | 22.0±3.0 |
| | (9SEL) *B. mojavensis 16S-gyrA*- | 27.0±1.0 |
| The hot spring ( Oued El Athmanya, Algeria): 1/1 | (SI) *B. amyloliquefaciens 16S-gyrA*-KC-204924 | 24.0±1.0 |

### Production of the Phytohormone Indole-3 Acetic Acid

The Salkowski reagent (0.01 M FeCl3 in 36 % H2SO4) was used to colorimetrically examine the production of indole-3 acetic acid (IAA) [21]. Isolates were grown in TGE (composition per liter: tryptone 5 g, glucose 10 g, beef extract 3 g) supplemented with 5 mM of L-tryptophan with agitation (160 rpm) at 30 °C for 4 days. Three hundred microliter of the Salkowski reagent was added to 100 μl of cultures in a microplate. After 15 min in the dark, color reaction intensity was estimated by measuring absorbance at 535 nm. The uninoculated Trp-containing medium mixed with the Salkowski reagent was used as blank. The concentration of IAA in each culture medium was determined by comparison with a standard curve [22, 23]. Results presented are means of two independent experiments.

### Production of Siderophores

Siderophore production was tested qualitatively using chrome azurol S medium (CAS-medium) as described by Husen (2003) [24]. Five microliter of each *Bacillus* suspension ($10^7$cellules/ml) were deposited on the surface of CAS agar medium and incubated at room temperature for 1 to 3 days. Siderophores production was indicated by yellow-orange halos around the colonies after incubation. This test was carried in three replications.

## Detection of Chitinase, Protease, and Cellulase Activities

Enzymatic activities were assessed in a qualitative way through halo formation on solid media containing colloidal chitin, milk powder, and carboxymethyl cellulose substrates to reveal successively chitinase, protease, and cellulase activities [25].

### Antagonism Tests on Root Exudates

In recent years, there is an increasing evidence of the root exudates importance in mediating the biological interactions between plants and microbes [26–28]. Indeed, the antifungal activity of some tested bacteria isolated in this work was investigated on root exudates. Tomato (*Solanum lycopersicum* cv. Tondo rosso), zucchini (*Cucurbita pepo* cv. Xara), and bean (*Proteus vulgaris* cv. Borlotto) root exudates were obtained by placing 20 sterile seedlings of each plant in 100 ml of Hoagland's solution and allowing them to grow in a growth chamber at 70 % relative humidity, 16 h of daylight, and 25 °C. After 15 days, root exudates were collected in 100-ml aliquots and conserved at −20 °C until use. Three *Bacillus* sp. (ET, 6SEL, and 9SEL) were tested for their ability to inhibit growth of *A. alternata* on root exudates plates' agar. The antagonism test was carried out in the same way as that described above.

### Effects of the Soil Treatment with the Biocontrol Agent (6SEL) on Chickpea Plant Size and Stem Rot Diseases; Under Greenhouse Conditions

Most of *Bacillus* biocontrol agents commercialized worldwide belong to *Bacillus subtilis*, *Bacillus pumilus*, and *B. amyloliquefaciens* spp. [29]. However, in this work, we chose the *Bacillus atrophaeus* (6SEL) strain to test its in vivo effect on chickpea plants under greenhouse conditions. This strain was produced under optimized industrial conditions in a 500-l bioreactor in the society Artechno SA (Belgium). The fermentation was stopped at the time of almost full sporulation, centrifuged and lyophilized to yield a highly concentrated stable powder. This

product was resuspended in sterile distilled water to obtain the final desired spore concentration ($10^7$ spores/ml). The chickpea seeds (cv. Flipe 13 90) were sown in a naturally infested soil with *Sclerotonia sclerotiorum*. The treatment was carried out by spraying the bacterial suspension on the soil and seeds. Two replicates were used; each replicate consisted of three pots (four seeds per pot). Size and stem rot rating (percentage of discoloration leaves per plant) data were recorded after 30 days of sowing. The SAS software (SAS Institute 2000) was used for all statistical analysis. The soil treatment effect on the studied parameters was assessed by a general linear model (GLM). Least square means (LSM) and standard errors (SD) were calculated, allowing ranking of treated and control lots according to Duncan's procedure.

## Results

### Antifungal *Bacillus* Strains Identification and Spore Yields

Fourteen *Bacillus* isolates recovered from extreme environments, i.e., the salt lake of Ain M'lila and the hot spring of Oued El Athmanya of Eastern Algeria. Seven of which (i.e., 50 %) displayed high antagonistic activity (Fig. 1) against various phytopathogenic fungi such as *F. oxysporum*, *B. cinerea*, *A. niger*, *C. cucumerinium*, and *A. alternata* (Table 2). The spores' yields of the screened isolates observed upon cultivation in agitated flasks varied between $2.2\times10^9$ and $2.7\times10^9$ spores/ ml (Table 1). The identification of these isolates based on *16S r-RNA* gene sequence analysis showed that they belonged to the *B. subtilis* spp. group, which includes several *Bacillus* sp. However, the comparison of their *gyr-A* gene sequences to those cited in GenBank by using the BLASTN program showed that 71 % of the isolates were identified as *B. amyloliquefaciens*, and the rest were closely related to *B. atrophaeus* and *B. mojavensis*.

### Antifungal Activity, Lipopeptides, and Cell Wall-Degrading Enzyme Production

The bacteria isolated in this work developed important fungal growth inhibition rates against *F. oxysporum* and *B. cinerea*, which were varying between 56 and 84 %. The

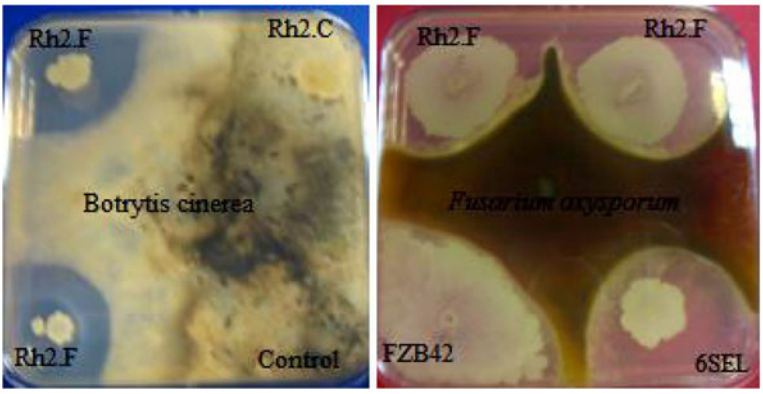


**Fig. 1** Antagonism test against developed by some tested bacteria (Rh2.C, Rh2.F, 6SEL, and FZB42), against phytopathogenic fungi Botrytis cinerea and Fusarium oxysporum. The antagonism test was achieved on potato dextrose agar (PDA) plates. The bacteria and the fungi were inoculated at the same time and the antagonism was scored after 2–5 days of incubation at 25 °C

**Table 2** Assessment of antifungal activity, lipopeptides, and cell wall-degrading enzymes production

| *Bacillus* isolates | Fungal growth inhibition (%) [a] | | Lipopeptides homologues production [b] | | | Cell wall-degrading enzymes production (clear zone in mm) | |
|---|---|---|---|---|---|---|---|
| | *F. oxysporum* | *B. cinerea* | Iturin | Fengycin | Surfactin | Protease activity [c] | Cellulase activity [d] |
| Rh2. A' | 72.5±2.5 | 74.2±1.4 | It A + | + | + | + | 1.3±0.3 |
| Rh2. F | 84.2±1.4 | 83.3 ±1.4 | It A + | + | + | + | 1.6±0.1 |
| ET | 70.8±2.9 | 74.2±1.4 | It A + | + | + | + | 1.8±0.1 |
| SEL | 65.8±1.4 | 75.0 ±2.5 | – | + | + | + | 1.5±0.1 |
| SI | 71.7±1.4 | 75.0 ±2.5 | It A + | + | + | + | 1.4±0.1 |
| 6SEL | 66.7±1.4 | 69.2 ±1.4 | – | – | + | – | – |
| 9SEL | 65.8±2.9 | 60.8 ±3.8 | – | – | – | – | 2.9±0.2 |

[a] Antagonism test on PDA plates, data were expressed as the percentage of mycelium expansion reduction, compared to control plates without bacteria

[b] Lipopeptides homologues produced by *Bacillus* strains after 72 h of growth in Opt medium, identified by ESI/LC-MS. *It A* iturin A, *It B D* iturin Bacillomycin D

[c] In vitro protease activity + present and − absent

[d] Carboxymethyl cellulose zone lyses diameter (cm). [a] and [d] data represent means ± standard errors

LC-ESI-MS analysis of supernatants showed that most of *B. amyloliquefaciens* strains produced the three LPs families of fengycin, iturin, and surfactin. However, the *B. atrophaeus* (6SEL) produced only surfactin and the *B. mojavensis* (9SEL) did not produce any type of LPs. A wide variety of homologous compounds were detected within each group of LPs, i.e., iturin A C-14 to C-17, surfactins C-12 to C-17, and fengycins (A) C-14 to C-19. For the cell wall-degrading enzymes production, protease and cellulase activities were observed in most of the *B. amyloliquefaciens* sp. However, chitinase production was only observed in the *B. atrophaeus* isolate (Table 2). Interestingly, besides the most common forms of fengycins homologues usually produced by *Bacillus* isolates, some additional picks were detected in the supernatant of the *B. amyloliquefaciens* isolates (Rh2.A', Rh2.F, ET, and SI). Several m/z of protonated fengycins were observed, i.e., 1,461.9 with −2 Da; 1,492.9 and 1,464.9 with +1 D; 1,495.9 and 1,481.9 with +4 Da (Fig. 2).

## In Vitro Production of Indole-3 Acetic Acid and Siderophores

All the *Bacillus* sp. studied here were able to produce IAA and siderophores. The *B. amyloliquefaciens* (*Rh2.A*) produced higher concentrations of IAA than that produced by the type strain FZB42. This strain in turn produced similar high levels of siderophores as the *B. amyloliquefaciens* strains *Rh2.A*', *Rh2. F*, and *ET* (Table 3).

## Antagonism Developed by the *Bacillus* Isolates Against *A. alternata* on Tomato, Zucchini, and Bean Root Exudates-Agar Plates

In general, the *Bacillus* strains, i.e., ET, 6SEL, and 9SEL, were able to inhibit the growth of *A. alternata* on root exudates of tomato, zucchini, and bean. The best antagonism was developed by the ET and 6SEL isolates on tomato root exudates with

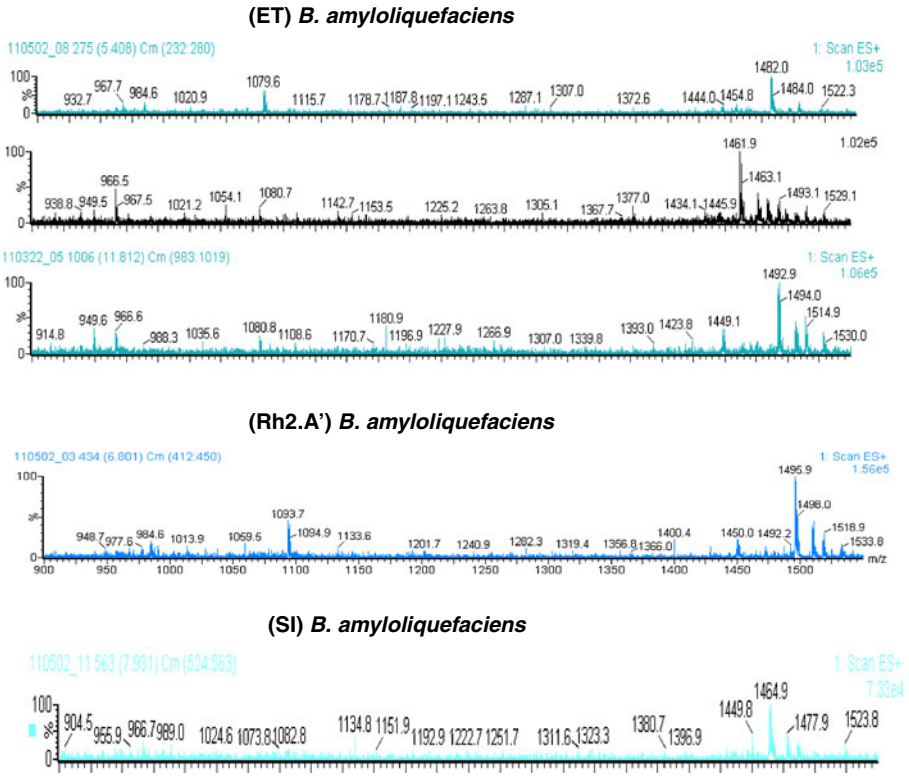


**Fig. 2** Some additional peaks with new masses compared to those of the most known protonated fengycin homologues, observed in LC-ESI.MS spectrum of *Bacillus amyloliquefaciens* strains isolated from extreme environments of Eastern Algeria

fungal growth inhibition reaching, respectively, 56 and 54 %. In contrast, the *B. atrophaeus* isolate (6SEL) did not inhibit the growth of *A. alternata* on root exudates of bean (Fig. 3).

**Table 3** In vitro determination of PGP traits in *Bacillus* isolates: production of indole-3-acetic acid (IAA) and siderophores

| *Bacillus* strains | IAA (μg/ml)[a] | Siderophores production (yellow-orange zone in mm)[b] |
|---|---|---|
| (Rh2. A') | 6.5±0.7 | 11.3±1.2 |
| (Rh2. F) | 6.5±0.7 | 11.0±1.0 |
| (ET) | 7.5±0.7 | 10.00±2.00 |
| (SEL) | 9.8±1.8 | 11.0±1.0 |
| (SI) | 6.5±2.8 | 8.7±1.2 |
| (6SEL) | 9.5±0.7 | 8.7±1.2 |
| (9SEL) | 11.5±0.7 | 8.7±1.2 |

[a] Colorimetric scale of IAA production

[b] In vitro siderophores activity, tested on Chrom azurol S medium plates. Data represent means ± standard errors

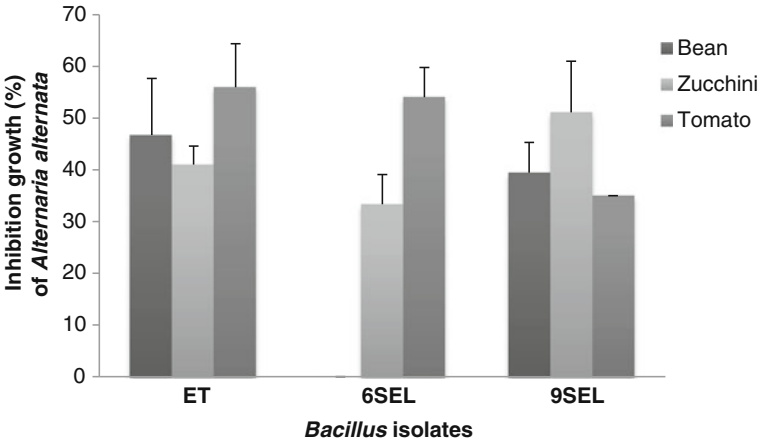


**Fig. 3** The antagonism developed by the three isolates: *B. amyloliquefaciens* (*ET*), *B. atrophaeus* (*6SEL*), and *B. mojavensis* (*9SEL*) against *Alternaria alternata* on root exudates of tomato, zucchini, and bean

## Greenhouse Experiment

The tested *B. atrophaeus* (6SEL) strain was efficient to promote the growth of chickpea plants and protect them against the phytopathogenic fungus *S. sclerotiorum*. Thus, the plants were significantly ($P<0.05$) higher with reduced stem rot rating in the treated pots in comparison with the control ones (Fig. 4).

## Discussion

Strains of the genus *Bacillus* are among the most commonly reported biocontrol and PGP rhizobacteria [6]. The characterization of *Bacillus* strains for their plant growth promotion and

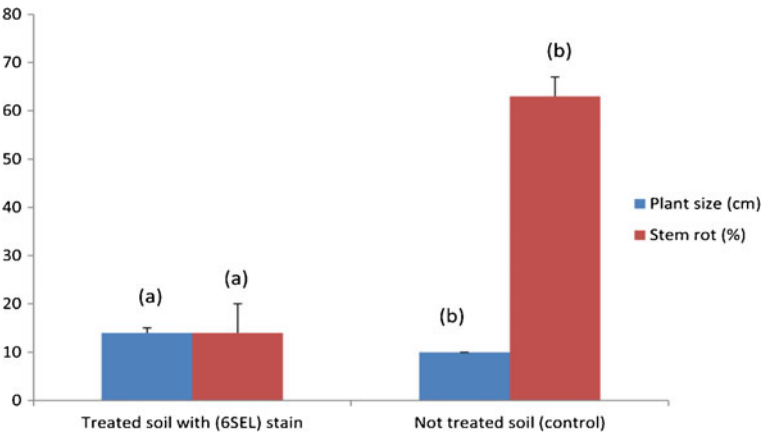


**Fig. 4** The effect of the *B. atrophaeus* (6SEL) on the size of chickpea plants and the stem rot disease rating after 1 month of seeds sowing. Different letters above histograms corresponding to the same parameter mention that the control pots are significantly different from the treated ones ($P<0.05$)

biocontrol capacities have been done widely on *Bacillus* strains isolated from the rhizosphere of agricultural crops [11, 22, 23]. However, this study is, to our knowledge, the first to study *Bacillus* strains isolated from extreme environments, i.e., the salt lake of Ain M'lila and the hot spring of Oued El Athmanya of Eastern Algeria. The eight screened *Bacillus* isolates developed important antagonistic activity against various phytopathogenic fungi. Interestingly, the *Bacillus* isolates tested here were characterized by high spore yields ($2.2\times10^9$–$2.7\times10^9$ spores/ml), which were higher than those found in previous studies where spore yields in submerged optimized cultivation were estimated at $8.35\times10^8$ spores/ml [30]. This may be related to the fact that they have to cope with adverse conditions in extreme environment from which they had been isolated [13, 14]. In addition, the spores yield of the *B. atrophaeus* strain (6SEL) in the industrial fermentor of 500 l (society Artechno SA, Belgium) reached $8\times10^{10}$ spores/ml and $2\times10^{11}$ spores/g of lyophilized product (data not shown). The interest of an efficient sporulation rate lies in the fact that it favors conditioning as powder formulations without dramatic bacterial mortality thereby representing a valuable technological advantage [5]. The identification of these bacteria using *16S r-RNA* gene sequences analysis was insufficient to discriminate between *Bacillus* sp. belonging to the *B. subtilis* spp. group because the average nucleotides similarity values of *16S r-RNA* sequences was 99.1 %. However, *gyr-A* gene sequences analysis were efficient to further clarify the identification of the *Bacillus* isolates. This result was similar to that previously found [31]. The *Bacillus* strains isolated here from extreme environments belonged to the *B. subtilis* spp. group. The *Bacillus* strains isolated from these same environments in several other studies were identified by only analyzing *16S r-RNA* sequences. These studies suggested that they belonged to divers *Bacillus* spp. Such as *Bacillus firmus* and *Bacillus mojavensis* [32, 33], *Anoxybacillus flavithermus*, *Geobacillus stearothermophilus*, and *Bacillus licheniformis* [34].

A relatively wide range of antagonistic performances among *Bacillus* strains was observed in this study and was also noted in other studies involving the same or different fungi [35]. Several mechanisms have been proposed to explain the inhibition of fungal growth by *Bacillus* spp., including the production of antimicrobial molecules, the secretion of hydrolytic enzymes, the competition for nutrients, or the combination of mechanisms [36]. In this study, protease and cellulase activities were observed in most of the *B. amyloliquefaciens* sp. However, chitinase production was only observed in the *B. atrophaeus* isolate. In addition, most of *B. amyloliquefaciens* isolates in this work produced iturin (A) C-14 to C-17, surfactin C-12 to C-17, and fengycin (A) C14 to C-19. These molecules were known for their surfactant and antifungal properties with most active ones observed in fengycins [37, 38]. De Carvalho and Pedro (2010) [13] and Dib et al. (2009) [14] have investigated that the extreme environments are interesting resources for stress-resisting microorganisms with exceptional phenotypic and genotypic characteristics. In this work, the mass spectrum of the *B. amyloliquefaciens* isolates (Rh2.A', Rh2.F, ET, and SI) was characterized by some particular peaks (Fig. 2), including ions with m/z lower or higher than the protonated conventional fengycin homologues. The m/z with -2 Da may correspond to the presence of a double bond in the B OH fatty acid moiety, a structural feature that has been previously characterized. The peaks with an increase in mass of 1D may be attributed to a fengycin species in which a Glu residue replaces a Gln residue at a position 8 in a lactone peptide cycle. The structure of +4 Da can be determined by further ESI-MS/MS analysis [39]. Consequently, the *B. amyloliquefaciens* isolates in the present study can be added a very limited number of strains co-producing fengycin homologues with such diversity [17]. Some of the antifungal strains isolated in this work including *B. atrophaeus* (6SEL), *B. mojavensis* (9SEL) and *B. amyloliquefaciens* (*RH2.A*) did not produce fengycin and iturin LPs; this showed that the observed antagonism was caused by other antifungal compounds. Thus, in the case of *B. atrophaeus* (6SEL), the antifungal activity can be

explained by its ability to produce chitinase, which was shown previously to inhibit fungal growth [40].

Several species of *Bacillus* spp. have been reported to produce auxins, which have a positive effect on root growth and morphology. In this study, all tested *Bacillus* sp. produced IAA in vitro with various concentrations ranged from 7 to 26 μg/ml. The same concentrations range was found by Subhash Yadav et al. (2011) [41]. In the present study, all *Bacillus* sp. produced siderophores which are responsible for the chelating of Fe and siderophores production is one of the most important depriving the phytopathogenic fungi of it [11]. Some of the most complex chemical, physical, and biological interactions experienced by terrestrial plants are those that occur between roots and their surrounding environment of soil [42]. In this work, some tested *Bacillus* isolates (ET, 6SEL, and 9SEL) were able to grow and produce antifungal molecules on tomato, zucchini, and bean root exudates. Furthermore, the in vivo test carried here showed that the *B. atrophaeus* (6SEL) had a significant effect on the plant size and the stem rot rating ($P<0.05$). Thus, it increased the size of the chickpea plants (14±1 cm) and reduced the stem rating disease (14±6 %). However, the plants in the control pots were of 10± 0.5 cm with 63±4 % of stem rot rating. Other *Bacillus* sp. had approximate effect on chickpea plants, as mentioned by Abel-Monaim (2011) [43] where *B. subtilis* and *B. megaterium* sp. decreased the pre-germination-damping-off, and the stem rot rating disease of Giza variety of chickpea. To conclude, extreme environments in the East of Algeria constitute an important source for *Bacillus* strains having an interesting and particular biocontrol and plant growth promotion traits. Their high spore yields may allow them to be a feasible product that can be used further for improving the crop systems.

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