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Ascochyta blight (*Ascochyta fabae*) of faba bean (*Vicia faba* L.): phenotypic and molecular characterization, pathogenicity and *in vitro* biological control by *Bacillus* spp. and *Pseudomonas* spp.

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Abstract: Ascochyta blight is a major biotic constraint of faba bean (*Vicia faba* L.) production and productivity worldwide caused by *Ascochyta fabae*. No studies have been performed in Algeria to identify *A. fabae* isolates or to assess their pathogenicity, and therefore information on local isolates is insufficient. Herein, 14 isolates of *A. fabae* were obtained from faba bean samples and identified based on morphological traits and phylogenetic analysis of internal transcribed spacer sequences. All generated sequences have been deposited in GenBank and assigned accession numbers. Pathogenicity tests on faba bean plants revealed that all isolates produced necrotic lesions on aerial parts with variable intensity, classifying them as weakly, moderately, and highly aggressive. The *in vitro* antifungal activity of *Bacillus* and *Pseudomonas* strains against *A. fabae* isolates showed that *Bacillus siamensis* B8 and *Bacillus mojavensis* B31 had the highest suppressive potential against all isolates. Moreover, a negative correlation was found between the aggressiveness of *A. fabae* isolates and their susceptibility to biocontrol strains. This is the first report on the identification, pathogenicity and *in vitro* biological control of *A. fabae* isolates in Algeria. B8 and B31 could be further developed as promising biocontrol agents for the control of the ascochyta blight of faba bean.

Keywords: Ascochyta blight; *Ascochyta fabae*; biological control; *Vicia faba* L.; faba bean; pathogenicity

Abbreviations: Analysis of variance (ANOVA); Bayesian information criterion (BIC); hierarchical principal component classification (HCPC); hydrogen cyanide (HCN); internal transcribed spacer regions (ITS); Kaiser-Meyer-Olkin (KMO); maximum likelihood (ML); mycelial growth inhibition potential (I%); nutrient agar (NA); nutrient broth (NB); potato dextrose agar (PDA); principal component analysis (PCA); standard plate count agar (APHA); the 0-5 scale infection type (IT); the 0-9 disease scoring recommended by ICARDA (International Center for Agricultural Research in the Dry Areas (ICARDA); tris-acetate-EDTA (TAE); water agar (WA)

INTRODUCTION

Grain legumes, also known as pulses, are one of the most environmentally sustainable food choices due to their nitrogen-fixing properties, their contribution to reducing greenhouse gas emissions, their promotion of soil carbon sequestration, and the induction of fossil energy savings in the system by the reducing of nitrogen fertilizers [1,2]. They are cultivated all over the world mainly for human and animal consumption. Legumes are low in fat and high in protein (20-36%), dietary fiber, carbohydrates, vitamins and minerals, making them part of a balanced and healthy diet [3,4]. In an effort to raise public awareness of the benefits of pulses to human health and their contributions to food security, nutrition and sustainable agriculture, the United Nations declared 2016 as the International Year of Pulses (IYP) [https://iyp2016.org/]. Faba bean

(*Vicia faba* L.), also known as broad bean, is one of the cool-season grain legumes widely cultivated around the world. In 2020, Algeria ranked 18th in terms of faba bean production globally and 5th in the Mediterranean countries [5]. The crop occupied first place among pulses in terms of cultivation area, production, and yield in Algeria. The area harvested for faba bean is estimated at 39,849 ha, which is equivalent to about 39% of the total area devoted to legumes, with an annual production of 49,856.7 t and productivity of 1,251.1 kg/ha [5]. However, the production of faba bean fluctuated between 1961 and 2020, according to FAO data. This instability may be due to the susceptibility of faba bean to biotic (fungal diseases, viruses, soil-borne pathogens, parasitic weeds, insect pests, and nematodes) and/or abiotic (drought and heat) stresses [6,7] that are responsible for considerable yield losses in the crop [8]. Ascochyta blight is one of the major biotic constraints of faba bean production and productivity in the Mediterranean climate [9,10]. It is a foliar fungal disease caused by *Ascochyta fabae* Speg. and is widespread throughout the world [11-13]. *A. fabae* attacks all aboveground parts of the plant (leaves, stems, pods, and grain), reducing seed quality and causing yield losses [14,15]. The gravity of the disease varies depending on the type of cultivar grown, infestation density, and environmental conditions. Yield losses of 35-40% are common [9,16]. In severe cases, yield losses can reach 90% when susceptible faba bean cultivars are grown and climatic conditions are conducive to disease development (cool and wet) [13,17,18].

During a heavy infection, stems may break at the site of lesions, resulting in lodging or plant death [19]. Possible damage caused by ascochyta blight includes reduced photosynthetic area, lodging due to stem girdling, abortion of pods and seeds, and seed infection [20]. Management of ascochyta blight can be achieved using one or some combination of the following main strategies: fungicide application as a seed treatment or by foliar spray, disease-free seed, cultural practices, manipulation of sowing dates, crop rotation and/or adoption of cultivars with improved resistance [9,10,18]. Nevertheless, these measures are only moderately effective, and each has deficiencies. Resistant cultivars are the best method of control, but their availability, the complexity of their traits and the high variability between *A. fabae* populations that changes the response of previously resistant lines, make them limited [21]. Chemical control has many disadvantages such as the high cost of fungicides both economically and environmentally; in addition, intensive and uncontrolled application of fungicides can cause the development of fungal strains resistant to commercial chemicals [22,23]. Faced with these concerns and the need for society to adopt environmentally friendly agricultural practices, alternative, more sustainable and ecologically relevant control strategies have been considered. Strains belonging to the genera *Bacillus* and *Pseudomonas* are among the most common biocontrol agents: their potential to inhibit phytopathogens causing crop diseases in laboratory experiments and/or under greenhouse conditions is very promising [24-26]. The ability of these bacteria to act as biological control agents is related through different mechanisms, including the production of siderophores, antibiotics, volatile compounds such as hydrogen cyanide (HCN) and other bioactive compounds, the induction of systemic resistance in the plant or the degradation of virulence factors of plant pathogens [27,28]. In previous work, a collection of strains of *Bacillus* and *Pseudomonas* was isolated from plant waste-based compost and screened for their antagonistic activities against several plant pathogens such as *Xanthomonas campestris* pv. *vesicatoria*, *Pythium ultimum*; *Fusarium oxysporum* f. sp. *melonis*, *Rhizoctonia solani*, *Phytophthora capsici* [29,30]. Considering that the biological control of *A. fabae* has not been previously addressed in the literature, the objectives of this work were: (i) to isolate and identify *A. fabae* from faba bean grown in northwestern Algeria; (ii) to study their pathogenicity on faba bean, which allowed us to divide them into groups according to their degree of aggressiveness; (iii) to evaluate the potential of 14 bacteria (4 *Pseudomonas* spp. and 10 *Bacillus* spp.) against *A. fabae* isolates, and (iv) to investigate whether there is a correlation between the aggressiveness of these isolates and their susceptibility to bacterial strains.

MATERIALS AND METHODS

Ethics statement

This article does not contain any studies with human participants or animals.

Sampling, isolation and morphological characterization of *A. fabae*

Strains of *A. fabae* were isolated from faba bean (*Vicia faba* L.) with typical ascochyta blight symptoms collected from different regions of northwestern Algeria during 2016 and 2017. Pieces of diseased plant tissues were superficially disinfected with a 10% sodium hypochlorite (NaOCl) solution for 5 min [31], rinsed three times in sterile distilled water, and dried on sterile filter papers. Pieces of plant tissues were then placed on potato dextrose agar (PDA) plates and incubated at 22-24°C under alternating cycles of light and darkness (12 h photoperiod) for 7 to 10 days. Single-spore isolates were prepared from selected colonies and stored as mycelial colonies on PDA slants at 4°C for further study.

A set of isolates presumed to belong to the *Ascochyta* genus were subjected to morphological studies such as colony characteristics (color, pycnidia production, and growth rate), pycnidial and conidial features (septal number of conidia, color, shape, and size). For each isolate, the length and width measurements of 30 randomly selected conidia were determined using a microscope camera (Leica) and ImageJ software. Growth rate (mm day^{-1}) was calculated by measuring the diameter of fungal colonies at 5, 7 and 10 days after inoculation with a mycelium plug for each of the three PDA plates per isolate.

Molecular identification of phytopathogenic fungi

DNA extraction

Fungal DNA of each isolate was extracted using Plant DNAzol® Reagent according to the manufacturer's protocol with some modifications (Invitrogen, Thermo Fisher Scientific corporation, Waltham, MA, USA). All isolates were initially grown on PDA plates for 10 days at 25°C. After incubation, fungal mycelia were harvested by scraping the culture using a sterile scalpel; a small amount of mycelia was transferred into a sterile microcentrifuge tube containing 150 µL of DNAzol. The mixture was shaken thoroughly, incubated for 5 min at room temperature and mixed 2 or 3 times during incubation by inverting the tube. After centrifugation at 10,000×g for 10 min, 112.5 µL of 100% ethanol was added to the resulting supernatant and centrifuged again at 7,000×g for 5 min. The supernatant was discarded and the pellet was washed with 150 µL of a mixture of DNAzol®/100% ethanol (1:0.75, v/v). The sample was maintained for 5 min at room temperature and then centrifuged at 7,000×g for 5 min. After discarding the upper aqueous layer, the pellet was washed with 150 µL of 75% ethanol followed by centrifugation at 7,000×g for 5 min. The DNA pellet obtained was subsequently air-dried, resuspended in 50 µL of pure water and incubated at 65°C in a water bath for 10 min. The crude DNA extracts were stored at -20°C until used for polymerase chain reaction (PCR).

Amplification and sequencing of nrDNA ITS regions

The internal transcribed spacer regions (ITS) region of the ribosomal DNA (rDNA) were amplified by PCR using the universal fungal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) according to White et al. [32]. The PCR experiments were performed in a final volume of 20 µL, containing 10 µL of 2X BioMix™ Red (Bioline, Meridian BIOSCIENCE® Memphis, TN USA), 0.4 µL of each primer, 7.2 µL of ultra-pure water and 2 µL of template DNA. Amplification was carried out in a MyCycler Thermal Cycler (Bio-Rad Laboratories, Inc. Berkeley, CA, USA) programmed as follows: 94°C for 10 min, 30 cycles of (94°C for 1 min, 50°C for 1 min, and 72°C for 3 min) and a final extension of 72°C for 10 min. To confirm the successful amplification, PCR products (5 µL of each amplicon) were analyzed by electrophoresis on 1% agarose gel in 1x TAE buffer (Tris-acetate-EDTA) stained with 0.004% GelRed® Nucleic Acid Gel Stain (Biotium San Francisco, CA, USA) and visualized under UV illumination in Gel Documentation system Gel Doc™ XR (Bio-Rad). Hyperladder™ 1 kb (Bioline) was used as a molecular size marker to estimate the PCR product sizes. The amplicons were purified using the mi-PCR Purification Kit (Metabion International AG, Planegg/Steinkirchen, Germany) following the manufacturer's instructions and then submitted for sequencing by capillary sequencer ABI Hitachi 3500 Gen Genetic Analyzer (Applied Biosystems, Thermo Fisher Scientific Corporation, Waltham, MA, USA).

Phylogenetic analysis of ITS sequences

The resulting ITS rDNA sequences were edited with the BioEdit Sequence Alignment Editor version 7.2.5 [33] and compared with those of references deposited in the GenBank database using the BLAST search of the National Center for Biotechnology Information (NCBI) [https://blast.ncbi.nlm.nih.gov/] to determine the closest species match. In addition to this, a set of 15 reference sequences retrieved from GenBank were included in the analysis (Table 1). All sequences were aligned using CLUSTAL W [34] and cleaned by Gblocks v.0.91b [35] to remove poorly aligned positions and highly variable regions of alignment. The phylogenetic tree was rooted using *Mycosphaerella punctiformis* isolate CBS 724.79 (GenBank accession No. AY490760) as the outgroup [36,37] and constructed by the maximum likelihood (ML) method with MEGA version 10.1.7 [38] according to the model of Kimura 2-parameter (K2) [39] with a proportion of invariable sites (+I). This model was selected as the best-fit nucleotide substitution model among 88 models tested in jModelTest v2.1.10 [40] based on the lowest Bayesian information criterion (BIC) score obtained. The reliability of a phylogenetic tree was assessed by bootstrap test with 1000 replicates. A generated phylogenetic tree was viewed using FigTree version 1.4.4 [http://tree.bio.ed.ac.uk/software/figtree/]. ITS sequences generated in this study were submitted to the GenBank database and accession numbers were obtained (Table 1).

Table 1. Strains of *Ascochyta* and *Mycosphaerella* species used for phylogenetic tree construction and their GenBank accession numbers. Algerian *A. fabae* isolates were used for all tests, including phylogenetic analysis, pathogenicity and *in vitro* susceptibility to bacterial antagonists.

Species	Isolate code	GenBank accession No.	Host	location
		ITS		
Ascochyta fabae	**A0**	**MN640736.1**	***Vicia faba***	**Algeria**
Ascochyta fabae	**A1**	**MN640737.1**	***Vicia faba***	**Algeria**
Ascochyta fabae	**A2**	**MN640738.1**	***Vicia faba***	**Algeria**
Ascochyta fabae	**A3**	**MN640739.1**	***Vicia faba***	**Algeria**
Ascochyta fabae	**A4**	**MN640740.1**	***Vicia faba***	**Algeria**
Ascochyta fabae	**A6**	**MN640741.1**	***Vicia faba***	**Algeria**
Ascochyta fabae	**A7**	**MN640742.1**	***Vicia faba***	**Algeria**
Ascochyta fabae	**A10**	**MN640745.1**	***Vicia faba***	**Algeria**
Ascochyta fabae	**A17**	**MN640748.1**	***Vicia faba***	**Algeria**
Ascochyta fabae	**A21**	**MN640751.1**	***Vicia faba***	**Algeria**
Ascochyta fabae	**A22**	**MN640752.1**	***Vicia faba***	**Algeria**
Ascochyta fabae	**A23**	**MN640753.1**	***Vicia faba***	**Algeria**
Ascochyta fabae	**A24**	**MN640754.1**	***Vicia faba***	**Algeria**
Ascochyta fabae	**AF**	**MN640755.1**	***Vicia faba***	**Algeria**
Ascochyta fabae	CBS 524.77	GU237880.1	*Phaseolus vulgaris*	Belgium
Ascochyta fabae	PD 83/492	GU237917.1	*Phaseolus vulgaris*	The Netherlands
Ascochyta fabae	CBS 649.71	MH860291.1	*Vicia faba*	The Netherlands
Ascochyta lentis	RGM_2731	MK934594.1	*Lens culinaris*	Chile
Ascochyta lentis	MU AL1	AY131201.1		/
Ascochyta viciae-pannonicae	CBS 254.92	KT389485.1	*Vicia pannonica*	Czech Republic
Ascochyta medicaginicola	CBS 112.37	MH855844.1		United Kingdom
Ascochyta medicaginicola	CBS 111.53	MH857116.1	/	USA: Minnesota
Ascochyta medicaginicola	CBS 479.63	MH858327.1	/	Italy
Ascochyta pisi	CBS 126.54	MH857263.1	*Pisum sativum*	The Netherlands
Ascochyta pisi	CBS 122785	NR_135981.1	*Pisum sativum*	The Netherlands
Ascochyta rabiei	CBS 581.83B	MH861657.1	*Cicer arietinum*	Syria
Ascochyta rabiei	CBS 206.30	MH855117.1	/	/
Ascochyta rabiei	CBS 534.65	MH858705.1	*Cicer arietinum*	India
Mycosphaerella punctiformis	CBS 742.79	AY490760.1	*Tilia sp.*	Germany

Newly generated sequences are indicated in bold; CBS – Centraalbureau voor Schimmelcultures (Central Bureau for Fungal Cultures), Utrecht, The Netherlands.

Bacterial antagonists and growth conditions

Fourteen antagonistic bacteria were selected from a collection of strains supplied by the group of Dr. Moreno (University of Almería, Spain), isolated from a composting process based on vegetable waste in 2015 (Table 2). The selection of these strains was based on their ability to inhibit other phytopathogenic agents, and their capacity to produce secondary metabolites such hydrogen cyanide, siderophores, salicylic acid, phosphates solubilization, and as chitinase-like enzymes [29,30]. Bacteria were stored on nutrient agar slants (NA; CM0003, Oxoid Ltd., UK) at 4°C, and cultivated in standard plate count agar (APHA) (Cutimed, Spain) for 24 h at 30°C and then in nutrient broth (NB; CM0001, Oxoid Ltd. UK) at 30°C for 24 h prior to use.

In vitro susceptibility of *A. fabae* isolates to bacterial antagonists

Four *Pseudomonas* and 10 *Bacillus* strains were screened for their antagonistic activity against 14 isolates of *A. fabae* by dual culture assay using the modified techniques of Landa et al. [41]. To perform the antagonism test, 2% water agar (WA) plates were prepared and used as the first layer. After solidification of the medium, 4 steel cylinders with a diameter of 8 mm were placed equidistantly along the periphery of the plate. A second layer of PDA was poured on the bottom agar (WA). The cylinders were removed when the PDA solidified. One 8-mm mycelial plug from the edge of a 10-day-old culture of *A. fabae* on PDA was inoculated in the center of the plate and precultured for 1 day at 25°C. After incubation, a liquid culture of the antagonistic bacteria (80 µL) was added to each well. A plate inoculated only with the phytopathogenic agent served as a control. The plates were prepared in 3 replicates and incubated for 11 days at 25°C. After incubation, mycelial growth inhibition potential (I%) was calculated using the following formula:

$$I\% = [(C-T)/C] \times 100$$

where C is the colony diameter of *A. fabae* in the control (mm) and T is the colony diameter of *A. fabae* in presence of the antagonistic strain (mm).

Table 2. Properties of the fourteen selected bacterial strains.

			Substance production [1]				
New code	Code	Phylogenic Affiliation	CHIT	CYAN	SID	SAL	PHO
B3	2296	*Bacillus aerophilus*	-	+	+	-	+
B4	2265	*Bacillus subtilis subsp. subtilis*	-	-	-	+	-
B5	2303	*Bacillus aerophilus*	-	-	+	-	+
B6	2082	*Pseudomonas rhizosphaerae*	+	+	+	+	+
B7	2298	*Bacillus megaterium*	-	-	+	-	+
B8	2727	*Bacillus siamensis*	-	-	+	-	-
B9	2668	*Pseudomonas xanthomarina*	+	+	-	-	-
B10	3358	*Bacillus aerius*	+	-	+	+	-
B11	2092	*Pseudomonas xanthomarina*	-	-	+	-	-
B20	2416	*Pseudomonas xanthomarina*	-	-	-	+	-
B28	2167	*Bacillus tequilensis*	+	-	-	+	-
B31	3654	*Bacillus mojavensis*	-	+	-	-	-
B32	2624	*Bacillus safensis*	-	-	+	-	-
B33	2788	*Bacillus aerophilus*	-	-	+	-	+

[1]CHIT – Chitinases; CYAN – cyanide; SID – siderophores; SAL – salicylic acid; PHO – phosphate solubilization

Pathogenicity assay – plant material and growth conditions

The pathogenicity test of 14 *A. fabae* isolates was performed on the faba bean cultivar Aguadulce following the protocol of Kaiser et al. [12] with some modifications. The choice of this cultivar is because it is one of the most cultivated varieties in Algeria according to the Technical Institute of Vegetable and Industrial Crops (ITCMI) [https://itcmi-dz.org/wp-content/uploads/2022/06/FEVE.pdf]. To obtain plants for the pathogenicity test, faba bean seeds were planted in 9-cm diameter pots (3 seeds per pot) filled with a steam-sterilized substrate mixture from an organic substrate (VLC Horticultura Cb, E98581028) and vermiculite (Projar, SA) at a ratio of 3:1 (v:v). The pots were placed in a growth chamber at 25°C.

Inoculum preparation and plant inoculation

Isolates of *A. fabae* were cultured on PDA plates and incubated at 22-24°C with a 12-h photoperiod for 10 days. Pycnidiospores of each isolate were harvested by flooding the surface of the PDA with sterile distilled water and scraping the culture using a bent glass rod. The conidial suspensions obtained were then

filtered through cheesecloth. Conidial concentration was determined using a Neubauer chamber and adjusted to $1x10^6$ conidia mL^{-1} before use as an inoculum. Plants at the 4-5 leaf stage were inoculated by spraying the suspension using a hand sprayer. Plants treated with sterile distilled water were used as negative controls. Three pots were used for each isolate (3 plants per pot). Immediately after inoculation, all plants were covered with plastic bags for 48 h to maintain humidity and kept at 25°C with a 12-h photoperiod.

Disease assessment

Disease development was assessed 15 days after inoculation using two rating scales as follows: (1) the 0-5 scale infection type (IT) on leaves [42]; (2) the 0-9 disease scoring recommended by the International Center for Agricultural Research in the Dry Areas (ICARDA) [20,43]. Disease severity scores were calculated as the average of 3 replicates per isolate. To fulfill Koch's postulates, the pathogen was re-isolated from infected tissues of inoculated plants with the technique described above and then identified by morphological characteristics.

Statistical analysis

All data analyses were performed in RStudio Version 1.3.959 [44] using R version 3.6.1. ANOVA was carried out using the data from the dual cultures and the pathogenicity assays. Prior to performing ANOVA, the data were tested for normality and homogeneity of variance using Shapiro-Wilk's and Leven's tests, respectively. When the null hypothesis of equal means was rejected, a post hoc Duncan's test was conducted in order to determine the significant differences among group means. Correlation analysis was performed using Pearson's correlation coefficient to describe the relationship between two normally distributed continuous variables. To check the suitability of the data for principal component analysis (PCA), the Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy and Bartlett's test of sphericity were computed. After performing the PCA, a hierarchical principal component classification (HCPC) was then applied to perform clustering using the complementarities between the clustering and principal component methods in order to better understand the data set. For all tests, P values less than 0.05 were considered statistically significant.

RESULTS

Morphological characterization and molecular identification

In the present study, 14 isolates were obtained from infected faba beans tissues (*Vicia faba* L.) collected in Algeria. All isolates were first identified to the genus level on the basis of morphological characterization and then subjected to molecular identification to confirm their identity. Morphological traits of the resulting isolates on PDA were noted after 10 days. Fungal colonies were initially whitish in color, later becoming light gray to dark brown with age from the center to the edge, and expanding 3.15 to 5.23 mm per day (at an average of 4.01±0.54 mm). All isolates produced pycnidia, which were sub-globose to globose, yellowish brown to dark brown in color, measuring 170-200×200-350 µm in diameter (Supplementary Fig. S1A). Pycnidiospores were hyaline, straight or slightly curved, rounded at both ends or slightly truncated at the base, mostly bicellular, but occasionally 2- to 3-septate. The conidial size ranged from 15 to 24 µm in length and 3 to 5 µm in width (Supplementary Fig. S1B, C). Morphologically, the characteristics of all isolates were similar to those of the *Ascochyta* genus.

In order to assign *Ascochyta* isolates to a particular species, molecular analysis was conducted using sequences obtained from the ITS 1 and 2 and 5.8S nuclear ribosomal DNA (nrDNA). The generated sequences were queried using BLAST against the GenBank nucleotide sequence database. BLASTn searches of ITS sequences from all isolates indicated 99.79-100% identity with corresponding sequences of *A. fabae* published in GenBank. Sequences derived in the present study were deposited in the GenBank database and their accession numbers are listed in Table 1. A total of 39 sequences, including 15 GenBank sequences, were used to construct a phylogenetic tree. The ML phylogenetic tree based on the ITS sequence data showed that the 14 sequenced isolates clustered together with the *A. fabae* reference strains available in the GenBank database: CBS 524.77, CBS 649.71 and PD 83/492 with a bootstrap value of 92% (Fig. 1).

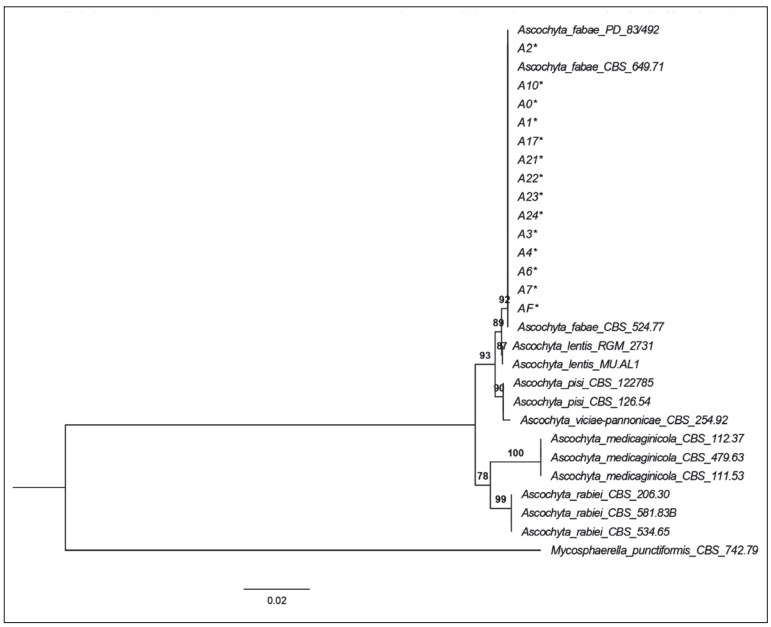

Fig. 1. Phylogenetic tree generated by the maximum likelihood method and K2+I model based on ITS sequences. The ITS sequences of 29 *Ascochyta* species retrieved from GenBank were included as references. The tree was rooted with *Mycosphaerella punctiformis* (CBS 742.79). Sequences generated in this study are marked by asterisks. The numbers above the branches represent maximum likelihood values based on 1K bootstrapped datasets. The scale bar indicates the number of nucleotide substitutions per site.

Pathogenicity test

The greenhouse study showed that isolates of *A. fabae* were capable of causing necrotic lesions on the aerial parts, leaves and stems, of inoculated plants but with varying degrees of severity. These symptoms were similar to those observed in the field. Lesions on the leaves start as small, circular, dark brown spots that enlarge to become circular or less regular in shape, slightly sunken with a chocolate brown or dark brown edge. At a later stage, a number of these spots coalesce under ideal conditions for the disease, covering a larger area of the leaf. As the disease progresses, the necrotic lesions develop fruiting bodies of the anamorph (pycnidia), which are small, raised black dots, arranged in concentric rings. Symptoms occur on the stems as dark spots become elongated and deeply sunken. The control plants remained healthy with no symptoms.

Depending on the isolates tested, the mean disease ratings using IT and ICARDA scales ranged from 0.3 to 5 and from 0.3 to 7.3, respectively (Table 3). The highest mean ratings for both scales were recorded for isolates A10 and A6, which produced the most severe symptoms on Aguadulce. On the other hand, the isolates A22, A23, A0 and A24 gave the lowest mean IT and ICARDA ratings as the mean disease scores were less than 3. The one-way ANOVA revealed significant differences in pathogenicity among the tested isolates (P<0.000).

The pathogens were successfully re-isolated from lesion tissues of inoculated plants and identified as *A. fabae* based on colony morphology and microscopic observations, which satisfied Koch's postulates. The results indicated that all 14 isolates of *A. fabae* were the causal agents of necrotic lesions on the aerial parts of faba bean cv Aguadulce. The two disease assessment methods (IT and ICARDA scales) were compared using Pearson's correlation coefficient. The correlation showed that IT ratings were strongly and positively correlated with ratings from the ICARDA scale (r=0.908, P<0.001).

Table 3. Pathogenicity test of *Ascochyta fabae* isolates on faba bean cultivar Aguadulce under growth chamber conditions.

Isolate	Disease assessment methods [a]	
	IT [1]	ICARDA [2]
A10	5 a	7 ab
A6	5 a	7.3 a
A7	4.6 a	6 abc
AF	4.6 a	6 abc
A3	4.6 a	5.6 abc
A17	4.6 a	5.3 bc
A21	4.3 a	5.3 bc
A2	4.3 a	5 c
A1	4 a	5 c
A4	4 a	5 c
A24	2 b	2.6 d
A0	1.6 b	1.6 de
A23	1 bc	1.3 de
A22	0.3 c	0.3 e

[a] Mean disease ratings (three replicate pots) assessed using two methods: IT and ICARDA scales [1]IT – Infection Type was scored according to the 0-5 scale; [2]ICARDA: the 0-9 disease scoring recommended by ICARDA; the means followed by different letters in the same column are significantly different (P<0.05) according to Duncan's test

Spectrum of *in vitro* inhibition

In order to determine the potential of bacteria to inhibit the growth of *A. fabae*, 14 bacterial strains were tested against 14 isolates of *A. fabae.* For this purpose, all *A. fabae* isolates were co-inoculated with each of the 14 bacterial strains (Supplementary Fig. S2). The ANOVA results showed significant differences between bacterial strains for their antifungal activities against *A. fabae* isolates in dual culture tests (Table 4).

Among the bacteria tested, both bacterial strains *Bacillus siamensis* B8 and *B. mojavensis* B31 demonstrated high inhibition activities against all isolates of *A. fabae* (I%>50% except for B8 against A6), with average inhibition rates ranging from 44.64% to 83.87% and from 51.75% to 77.78%, respectively (Table 4). Furthermore, B8 showed the highest inhibition effect on the percent mycelia growth of A24 (81.9%), A23 (80.36%), A0 (77.67%), A10 (74.42%), A21 (70.93%) and A1 (59%). The strains *Pseudomonas rhizosphaerae* B6, *P. xanthomarina* B9*, Bacillus tequilensis* B28 and *B. subtilis subsp. subtilis* B4 showed strong antagonistic activities (I%>50%) against at least eight isolates of *A. fabae*.

Two strains of *B. aerophilus* (B3 and B5) and one of *B. megaterium* (B7) significantly suppressed the growth of mycelia of 3 and 5 isolates of *A. fabae*, respectively, with inhibition rates exceeding 50%. Meanwhile, *B. aerius* B10 and *P. xanthomarina* B20 presented inhibition rates greater than 50% toward only one isolate, A22 and A24, respectively (Table 4, Fig. 2). In contrast, the remaining 3 bacterial strains, *P. xanthomarina* B11, *B. safensis* B32 and *B. aerophilus* B33, expressed the lowest inhibitory activity (<50%) against the collection of isolates tested, as shown in Fig. 2.

Regarding the isolates of *A. fabae*, the least susceptible isolates to the antagonistic bacteria assayed were the most pathogenic isolates (A6 followed by A10). In contrast, the weakly pathogenic isolates were more susceptible to all the biocontrol strains, except for those that presented the lowest inhibitory activity (*B. aerophilus* B33, *B. safensis* B32 and *P. xanthomarina* B11). Compared to the moderately and highly pathogenic isolates, A22 was the most sensitive and the least pathogenic. Moreover, A22 was fully inhibited by *B. subtilis subsp. subtilis* B4 (98.34%). The highest rates of mycelial growth inhibition of A4, A6 and AF were recorded by *B. mojavensis* B31, while those of A2 and A17; A1, A3 and A7; and A3 were observed by *B. subtilis subsp. subtilis* B4, *P. rhizosphaerae* B6 and *P. xanthomarina* B9, respectively.

A scatter plot was constructed to identify the most suppressive strains against *A. fabae* isolates, these strains being *B. siamensis* B8 and *B. mojavensis* B31 (Fig. 2). PCA is a multivariate statistical analysis technique that reduces the dimensionality of a data set by transforming a large number of intercorrelated variables into fewer dimensions called principal components (PCs) while preserving as much of the relevant information as possible. Its goal is to find the best summary and visualization of high dimensional data using a limited number of PCs [45] and to display the pattern of similarity of observations and variables in maps [46].

As shown in Fig. 3A, the first two principal components (PC1 and PC2) accounted for 53.63% and 16.84%, respectively, of the total variance in the original data. These two PCs were selected for performing PCA as they explained 70.74% of the total inertia and had individual eigenvalues greater

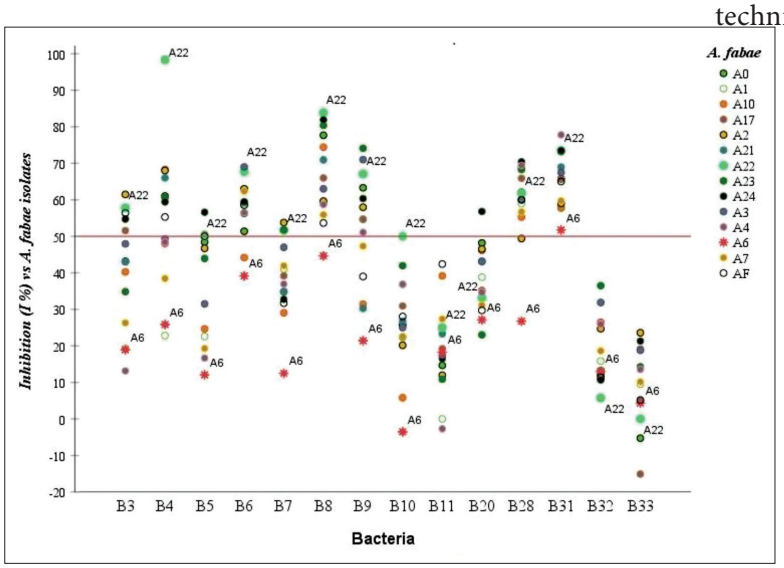

Fig. 2. Scatter plot for the biocontrol potential of fourteen selected bacterial strains to inhibit *Ascochyta fabae* isolates *in vitro*. Inhibition rates (I%) above 50% are marked in the diagram.

Table 4. Antagonistic activities of 14 selected bacterial strains against 14 isolates of *Ascochyta fabae*.

Ba*	Percent inhibition of radial growth of the pathogen by antagonistic bacteria (I%)													
New code	**A0**	**A1**	**A2**	**A3**	**A4**	**A6**	**A7**	**A10**	**A17**	**A21**	**A22**	**A23**	**A24**	**AF**
B3	43.16± 2.11 e	19.26± 2.38 ef	61.47± 0.82 bc	47.94± 5.48 de	13.15± 4.12 g	18.96± 1.73 e	26.31± 0.3 fg	40.26± 0.24 e	51.58± 0.54 cd	43± 0,89 e	57.78±2.23 f	34.85±1.52 h	54.72±0.76 e	56.39± 1 cd
B4	61.03± 1.93 c	22.81± 2.53 e	**68.03± 0.82 a**	49.31± 1.37 d	48.42± 1.59 d	25.86± 1.7 d	38.46± 1.77 d	48.05± 2.6 c	**68.32± 0.24 a**	66±0.7 b	**98.34±0.9 a**	60.6± 3.04 d	59.43±2.83 d	55.3± 1.47 cd
B5	48.42± 1.05 d	22.58± 2.68 e	46.72± 0.82 e	31.5± 1.62 f	16.67± 1.52 g	12.07± 1.73 f	19.3± 3.51 h	24.67± 2.63 h	50.53± 3.16 d	50±2 d	50± 1.11 g	43.94±1.52 g	56.6± 1.85 e	50± 3.19 e
B6	51.38 ± 4.26 d	**58.95± 1.06 a**	63.02± 1.68 b	**69±1 a**	56.52± 0.63 c	39.15± 0.63 c	**62.37± 1.08 a**	44.19± 0.2 d	62.89± 2.07 b	59.3± 3.49 c	67.74±2.15 d	56.25±0.89 e	59.48±0.86 d	58.54± 2.44 bc
B7	34.86± 0.92 f	41.05± 4.21 c	53.78± 0.84 d	47±1 de	36.95± 0.61 e	12.5± 1.78 f	41.94± 0.86 d	29.07± 3.49 fg	39.18± 3.1 e	34.88± 4.65 f	51.61±1.08 g	51.79±5.36 f	32.76±1.08 f	31.7± 2.07 h
B8	**77.67± 2.19 a**	**59 ± 0.98a**	59.66± 1.68 bc	63±1 bc	58.7± 2.18 c	44.64± 1.79 b	55.91± 1.03 b	**74.42± 0.52 a**	65.98± 1.03 ab	**70.93± 1.16 a**	83.87±1.13 b	**80.36±1.79 a**	**81.9± 0.81 a**	53.66± 0.7 d
B9	63.3± 1.84 bc	54.74± 1.06 b	57.96± 3.93 c	**71±1 a**	51.09± 1.09 d	21.43± 3.57 e	47.31± 0.98 c	31.4± 1.17 f	54.64± 0.65 c	30.27± 0.51 g	67.07±4.6 d	74.11±0.9 b	60.34±1.73 d	39± 1.91 g
B10	25.69± 0.92 g	36.84± 4.21 d	20.17± 2.52 g	25±5 g	36.87± 2.73 e	-3.53± 0.56 h	22.57± 1.67 gh	5.81± 1.18 j	30.93± 1.03 g	26.74± 1.17 h	50±3.38 g	41.96±2.68 g	22.37±0.79 g	28.05± 1.22 i
B11	14.65± 0.87 h	0±0 h	11.97± 2.57 h	17.39± 4.35 h	-2.7± 2.9 h	18.29± 1.33 e	27.37± 5.27 ef	39.18± 1.04 e	19.23± 3.85 h	23.33± 1.11 h	25± 0.92 i	10.87±2.18 j	16.52±1.64 h	42.4± 1.6 f
B20	48.19± 1.21 d	38.82± 0.8 cd	46.53± 2.28 e	43.08± 1.54 e	34.66± 2.01 e	27.14± 1.43 d	31.17± 3.9 e	46.15± 2.57 cd	35.2± 0.26 f	43.21± 0.84 e	33.3± 0.61 h	23.03±1.27 i	56.81±0.75 e	29.73± 2.03 hi
B28	62.04± 0.93 c	**58.97± 1.27 a**	49.4± 2.41 e	60±4.1 c	69.44± 0.93 b	26.77± 1.76 d	56.72± 1.5 b	55.26± 2.63 b	65.9± 0.6 ab	68.97± 3.45 ab	61.75±0.91 e	68.27±0.74 c	70.41±1.02 c	60±0.5 b
B31	65.74± 0.93 b	57.69± 1.28 ab	59.03± 2.52 c	67.5± 2.5 ab	**77.78± 1.32 a**	**51.75± 1.74 a**	59.7± 1.4 ab	57.89± 2.64 b	65.91± 2.27 ab	68.97± 1.15 ab	73.52±1.36 c	73.19±1.17 b	73.47±2.04 b	**65± 0.95 a**
B32	12.28± 3.51 h	15.87± 1.59 f	24.72± 1.12 f	31.88± 1.45 f	25.93± 3.54 f	13.04± 0.92 f	18.64± 3.39 h	26.47± 2.94 gh	5.66± 1.77 i	13.33± 0.5 i	5.77± 1.83 j	36.5± 0.5 h	10.67±1.34 i	11.4± 3.94 j
B33	-5.26 ± 1.59 i	9.52 ± 1.59 g	23.6± 2.25 fg	18.8± 0.89 h	13.58± 1.62 g	4.34± 0.59 g	10.17± 1.7 i	19.12± 1.47 i	-15.09± 2.52 j	5±1.67 j	0±1.18 k	14.26±1.87 j	21.33±1.53 g	5.17± 1.75 k

*Ba – bacterial antagonist. Data in the table are means±SD (SD – standard deviation; n=3 replicates). Different letters in the same column indicate values that are significantly different at P<0.05 according to Duncan's test; bold values indicate the highest inhibition rates

Table 5. Correlations between pathogen aggressiveness of *Ascochyta fabae* isolates and susceptibility to bacterial strains.

Pearson's correlation coefficient		
Biocontrol strains	Aggressiveness	
	IT	ICARDA
B3	-0.26	-0.30
B4	**-0.60**	**-0.63**
B5	-0.51	**-0.57**
B6	-0.21	-0.33
B7	-0.42	-0.51
B8	**-0.78**	**-0.76**
B9	**-0.66**	**-0.73**
B10	**-0.61**	**-0.68**
B11	0.21	0.28
B20	-0.03	-0.05
B28	-0.41	-0.47
B31	**-0.60**	**-0.63**
B32	0.10	0.12
B33	0.11	0.16

Marked correlations are significant at P<0.05

than 1.0. The PCA results were represented as a biplot in which variables were displayed as vectors and samples as points in the map. The data set consisted of 14 individuals corresponding to 14 isolates of *A. fabae* described by 17 variables. Of these, 14 variables represent the potential antagonism of the 14 bacteria towards various strains of *A. fabae* and 2 more variables are also quantitative, including the level of pathogenicity (evaluated by IT and ICARDA scores) of 14 isolates of *A. fabae* on faba bean. In addition, there is also a categorical variable with 3 categories: highly, moderately and weakly pathogenic.

Most of the variables contributed to the creation of PC1, which is why axis 01 had the highest variance (53.63%). The variables B4, B8, B31, B6, B28 and B9 are the most correlated (>0.6) and well represented on axis 01 (cos^2 near to 1). They are positively associated with PC1 and negatively correlated with IT and ICARDA scores. As shown in Table 5, a significant correlation negative was found between the sensitivity of *A. fabae* isolates to B8, B9, B10, B31 and B4 and their aggressiveness (scored by IT and ICARDA) based on Pearson's correlation coefficient.

The weakly pathogenic isolates A22, A23, A24 and A0 formed a distinct cluster on the right of the PCA by being strongly inhibited by B4, B8, B31, B6, B28

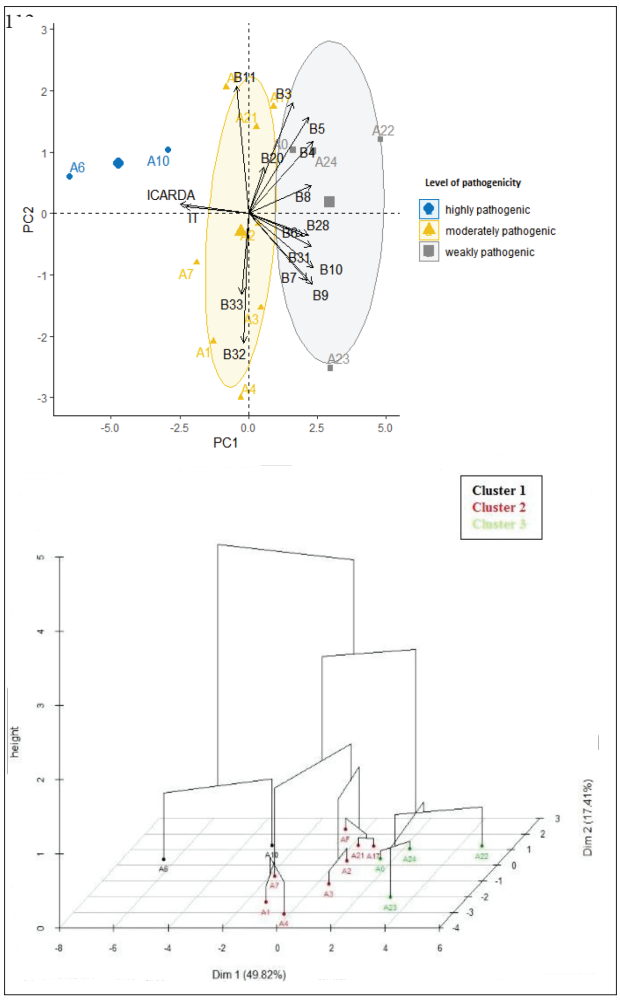

Fig. 3. A –Principal component analysis (PCA) of 14 *Ascochyta fabae* isolates based on pathogenicity and inhibition levels; 2D biplot generated by the first two components in which variables were displayed as arrows and isolates of *A. fabae* as points in maps. **B** – Three-dimensional representation of the hierarchical clustering on principal components (HCPC) on the map produced by the first two principal components; Cluster 1 grouped the most pathogenic and least inhibited isolates of *A. fabae*; Cluster 1 (black) – the most pathogenic and least inhibited isolates of *A. fabae*; Cluster 2 (red) – moderately pathogenic isolates; Cluster 3 (green) – the least pathogenic and most inhibited isolates.

and B9, resulting in this group of samples (A22, A23, A24 and A0) moving to this side. The second group included A1, A4, A2, A21, A17, A3, AF and A7, which were moderately pathogenic (4≤IT<5; 5≤ICARDA≤6) and were inhibited by at least 4 bacteria (I%>50%). The highly pathogenic isolates (A6 and A10) were found in a separate group to the left of PC1. This group was characterized as the least inhibited by most bacteria (I%<50%, except for B8, B28 and B31 against A10 as well as B31 *versus* A6) and had the highest IT and ICARDA scores.

The biplot generated by PCA shows 3 groups of *A. fabae* strains that are similar in terms of pathogenicity and inhibition (Fig. 3A). This result was also confirmed by a HCPC (Fig. 3B), which clearly delineated 3 distinct clusters of *A. fabae* strains according to their level of pathogenicity and inhibition by antagonistic bacteria.

DISCUSSION

To the best of our knowledge, this study describes for the first time the identification of *A. fabae* isolated from Algeria and their biological control by antagonistic bacteria. Fourteen strains of *A. fabae* were isolated from infected tissues of faba bean grown in various locations in the northwestern region of Algeria. The identification of the strains was done on the basis of morphological characteristics and molecular analyses [11,47].

Most morphological features are highly variable and have no specificity. Therefore, relying on these characteristics to differentiate species in *Ascochyta* is impractical. In contrast, the only characters considered taxonomically pertinent are the conidial shape and size [48]. The conidia of our isolates were cylindrical, straight or slightly curved, rounded at both ends or slightly truncate at the base, mostly with one septate, ranging from 15 to 24 µm in length and 3 to 5 µm in width. These characteristics are close to those of *A. fabae* [31,48,49]. Furthermore, the fungal isolates from the current study were identified at species level by sequencing of the ITS region and then subjected to BLASTn for the similarity search. These sequences were compared with 15 other reference sequences of *Ascochyta* species commonly infecting pulses that are available in the GenBank database. Phylogenetic analysis of the ITS sequences using the maximum likelihood method and K2+I model indicated that our isolates were monophyletic and formed a well-supported clade with sequences of *A. fabae* available online in the GenBank (PD 83/492, CBS 524.77 and CBS 649.71).

These results agree with a previously published study [11]. Peever [47] indicated that phylogenetic relationships of the combined glyceraldehyde-3-phosphate-dehydrogense, translation elongation factor alpha and chitin synthase datasets for *A. fabae*, *A. pisi*, *A. rabiei*, *A. viciae-villosae* and *A. lentis* were monophyletic with strict correlation

between the phylogenetic clade and host of origin. Phylogenetically, our isolates appeared to be related to *A. fabae* based on the similarity of their ITS nucleotide sequences. The combination of morphological characteristics and ITS sequence analysis allowed us to identify our isolates as *A. fabae*.

The pathogenicity test showed that all tested isolates reproduced aerial disease lesions on inoculated faba bean plants cv. Aguadulce under greenhouse conditions, with varying intensity assessed by the IT and ICARDA scores. These symptoms were identical to those originally observed in the field and were typical of those caused by *A. fabae* on faba bean plants [13,19]. The pathogens were successfully re-isolated from leaves and stems of inoculated plants and were morphologically and culturally identical to the original isolated fungus [31,48,49], confirming Koch's postulates.

The pathogenicity assay confirmed the infectivity of our isolates on faba bean and identified the causal agent of ascochyta blight as *A. fabae*. These results agree with a previous report, which demonstrated that *A. fabae* is the causal agent of ascochyta blight of faba bean [12]. Tivoli and Banniza [50] reported that the pathogens belonging to the genus *Ascochyta* are responsible for ascochyta blight of pulses. Hernandez-Bello et al. [11], Kaiser et al. [12], and Peever [47] found that *Ascochyta* fungi associated with pulses are host-specific.

The management of fungal and bacterial phytopathogens with *Bacillus* spp. and *Pseudomonas* spp. is a promising approach in biological control. Many reports exist in the literature about the effects of these bacterial antagonists in the control of phytopathogens of different crops *in vitro* and/or *in vivo*, but there are no studies about the potential of *Bacillus* spp. and *Pseudomonas* spp. to repress the growth of *A. fabae*. Different mechanisms are used by *Bacillus* and *Pseudomonas* species to suppress plant pathogenic microorganisms. These mechanisms could be related to the ability of biocontrol agents to produce a wide array of secondary metabolites, including volatile and non-volatile organic compounds (siderophores, hydrogen cyanide, etc.), hydrolytic enzymes (such as chitinases, glucanases, cellulases, xylanases and proteases) and other metabolites [28,52].

Previous studies focused on the isolation of bacteria from compost and the evaluation of their antagonistic effects against a wide range of phytopathogens such as *Xanthomonas campestris* pv. *vesicatoria*, *Pythium ultimum*; *Fusarium oxysporum* f. sp. *melonis*, *Rhizoctonia solani*, *Phytophthora capsici* [29,30]. In the current study, we selected 14 bacterial strains belonging to *Bacillus* and *Pseudomonas* genera from this collection and tested them against 14 isolates of *A. fabae* responsible for ascochyta blight on faba bean. *In vitro* antifungal activity of the selected biocontrol bacteria showed that most of them were able to significantly inhibit the mycelial growth of *A. fabae* isolates with varying degrees of effectiveness. Indeed, we found that *Bacillus siamensis* B8, a siderophore-producing bacterium, and *Bacillus mojavensis* B31, which produces cyanide, had the highest inhibitory activities against all isolates of *A. fabae,* making them the most potent antagonistic strains. Similar results were also found for these 2 strains against *Rhizoctonia solani* and *Phytophthora capsici*, with growth reduction levels above 75% [29].

In the competition for nutrients between bacteria and phytopathogens, especially under iron-deprived conditions, siderophores, which are iron-chelating compounds with a high affinity for ferric iron, could be synthesized by the beneficial bacteria in order to uptake iron, making it unavailable to pathogenic microorganisms and consequently limiting their growth [51]. Shen et al. [53] screened a siderophore-producing bacterium, *Bacillus siamensis* Gxun-6, against *Fusarium oxysporum* f. sp. *cubense* with Tropical Race 4 in both *in vitro* and *in vivo* tests. They showed that Gxun-6 exhibited an antifungal activity reaching 68.8% and a strong growth-promoting effect on banana.

Hydrogen cyanide (HCN) emitted by cyanogenic bacteria could play a crucial role in controlling some plant pathogens, as it is a respiratory toxin that has a potent inhibitory influence on the key respiratory enzyme, cytochrome C oxidase, and on the synthesis of several other metalloenzymes, causing deleterious effects to the pathogen [54]. The cyanogenic bacteria *P. putida* R32 and *P. chlororaphis* R47 provided overall activity against the mycelial growth of *Phytophthora infestan*s in dual culture trials [55,56]. According to Anand et al. [57], the total inhibitions of oomycetes in volatile-mediated dual assays were mainly or

solely due to the emission of HCN by the R47 and R32 strains, respectively. The high antagonistic ability of *Pseudomonas rhizosphaerae* B6 to inhibit twelve isolates of *A. fabae* in dual culture (direct action) at a rate of more than 50%, and its capacity to produce siderophores, chitinase, cyanide, salicylic acid and phosphate solubilization, places it as the top of biocontrol candidates among the *Pseudomonas* tested.

The aptitude of biocontrol strains to produce lytic enzymes such as chitinases, which are among the most widely studied, allows them to degrade and cause leakage of the fungal cell wall, exploiting the hydrolysis products as carbon source and thereby inhibiting the growth of pathogens [28,58]. Shali et al. [59] demonstrated that the two different chitinases secreted by *B. pumilus* SG2 were mainly responsible for growth inhibition of *Fusarium graminearum* and interrupted hyphal elongation of *Bipolaris sorokiniana*. Likewise, a chitinase from *Pseudomonas aeruginosa* GRC_1 appeared to be mediated in the control of *Sclerotinia sclerotiorum* by perforating, lysing and fragmenting its hyphae [60].

The difference in biocontrol potential shown among the fourteen selected bacteria could be linked to their capacity to produce one or more bioactive compounds with a concentration that may be variable, and/or the possession of different anti-*Ascochyta fabae* arsenals currently unknown. The most aggressive strains of *A. fabae*, A6 and A10, were less sensitive to biocontrol strains than the moderately and weakly pathogenic isolates. A negative correlation between pathogenicity and *in vitro* inhibition confirmed this finding, especially when co-inoculated with B8. This result agrees with those obtained by De Vrieze et al. [24], who found that the pathogen aggressiveness and virulence of *Phytophthora infestans* isolates were negatively correlated with the sensitivity to *Pseudomonas* strains particularly R76.

CONCLUSIONS

In our study, 14 strains of *A. fabae* collected from Algeria were isolated from faba bean showing ascochyta blight symptoms and fully identified by morphological criteria, such as colony characteristics, pycnidial and conidial features and growth rate, and molecular analysis. Pathogenicity tests fulfilled Koch's postulates and demonstrated that the isolates were the causal agent of ascochyta blight on faba bean, with varying degrees of pathogenicity (low, moderate and high). Knowledge of the characterization of these isolates will provide the basis for epidemiological studies and for the establishment of effective management strategies against ascochyta blight of faba bean, and may also fill gaps in the information on local isolates. In terms of biocontrol activity, *Bacillus siamensis* B8 and *B. mojavensis* B31 showed a high capacity to inhibit all *A. fabae* isolates, and this activity correlated negatively with the degree of pathogenicity. As there are no studies on the biocontrol of *A. fabae*, this paper provides preliminary research into the antagonistic potential of *Bacillus* and *Pseudomonas* species on *A. fabae* inhibition *in vitro*. The subject warrants further research to evaluate the actions of *Bacillus siamensis* B8 and *Bacillus mojavensis* B31 toward *A. fabae* in volatile compound-mediated dual assays, in the whole plant and under field conditions, to better understand the relationship between the aggressiveness of *A. fabae* and its susceptibility to biocontrol agents, with a view to proposing an alternative to synthetic fungicides.

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Conflict of interest disclosure: The authors declare no conflict of interest.

Data availability: All data underlying the reported findings are available in a publicly accessible repository. The data presented in this study are openly available in FigShare at https://doi.org/10.6084/m9.figshare.22009568.v1. All generated sequences in this study are available in the DDBJ/EMBL/GenBank databases under the accession numbers MN640736 to MN640742, MN640745, MN640748 and MN640751 to MN640755.

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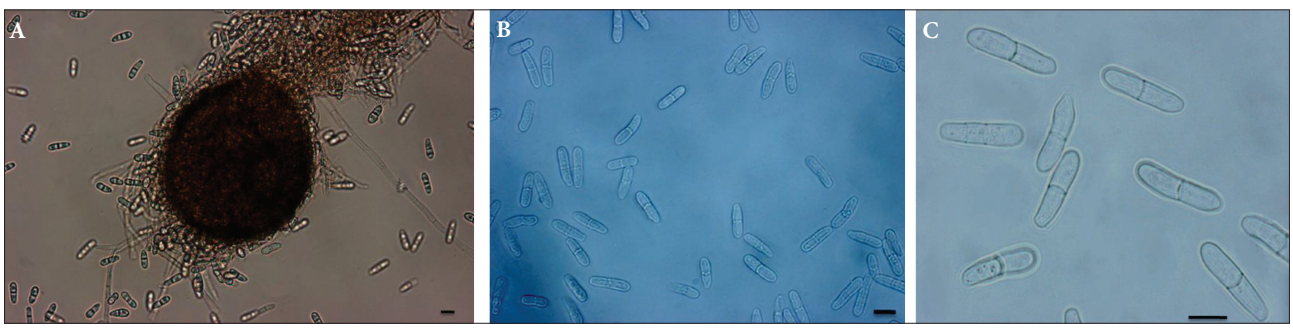
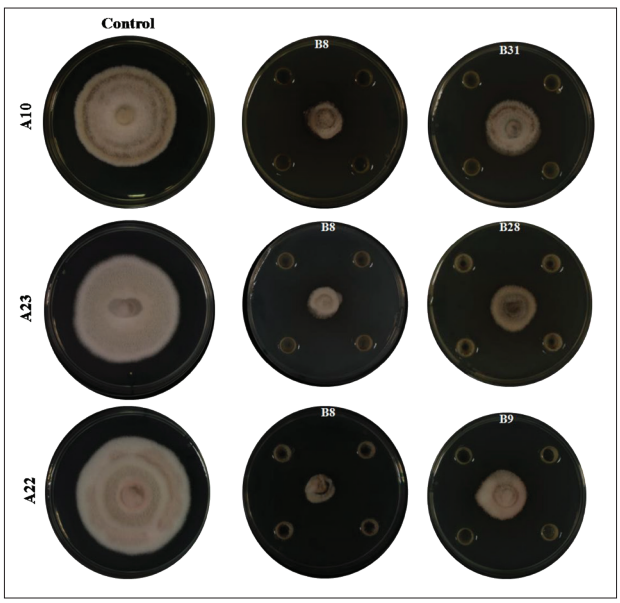
SUPPLEMENTARY MATERIAL



Supplementary Fig. S1. Micromorphology of *Ascochyta fabae*: **A –** Pycnidium asexual fruiting body; **B and C –** conidia. Scale bars: 10 µm.



Supplementary Fig. S2. Effect of bacterial antagonists on mycelia growth of *Ascochyta fabae* isolates in a dual culture assay.