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Pseudomonas diversity in western Algeria: role in the stimulation of bean germination and common bean blight biocontrol

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Abstract The aim of this work was to determine the functional diversity of soil bacteria belonging to the *Pseudomonas* genus, to study their effects on bean (*Phaseollus vulgaris* L) seed germination and their biocontrol potential of common bean blight. Bacteria were isolated and identified based on physiological and biochemical characters and BOX-PCR. Followed by qualitative and/or quantitative analysis of their secondary metabolites. 50 soil bacteria were affected to the two groups of fluorescent (72%) and non-fluorescent Pseudomonads (28%). The UPGMA (Unweighted Pair Group Method with Arithmetic Mean) showed five phenons of carbon sources assimilation; at the time that BOX-PCR profiling resulted in five clusters characterized by 29 different haplotypes. (66%) isolates induced phosphate solubilization; (24%) were HCN producers, (21%) showed IAA production and all isolates had produced siderophores. In vitro antibacterial activity against Xapf showed 26.67 and 24 mm of inhibition zone using the two isolates *P. grimontii* P25 and *P. cepatia* P7, respectively. Similarly, the same isolates significantly reduced Xapf-bean common blight intensity, while their co-inoculation was less effective. The isolate *P. cepatia* P7 was highly effective on seed germination and root growth properties, then *P. grimontii* P25. Thus, the selected isolates could play a crucial bean growth promotion and bean common blight biocontrol as alternative to chemicals for crop yield enhancement.

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Introduction

Nutritional problems touch about 2 billion people worldwide **(**Hawkes and Ruel 2008). In addition, the world's population, estimated around 7 billion people at this time, is predicted to jump to around 10 billion in the next 50 years, which requires significant increase in food productivity human life continuity on the planet (Glick 2014). In this context, agricultural practices are the major source of food and feed around the world. Without enough agricultural production, catastrophic scenarios are objectively expected in the next few years (Giovannucci et al. 2012).

Modern agriculture is facing major challenges because of climate changes, increasing world population, natural resources scarcity and food transitions to more meat products (Urruty 2017). Thus, the produced crop need to be equipped with disease eradication systems, salt-, drought-, heavy metal-tolerance characters, and better nutritional values (Armada et al. 2014). In some regions of the globe, up to 30% agricultural yield is lost every year due to both abiotic and biotic stresses. These last are the result of damages done to plants by other living organisms such as bacteria, viruses, fungi, parasites, insects, weeds, and cultivated or native plants (Touraev and Jones 2015).

Soil physical and chemical properties control (temperature, soil salinity, moisture, etc.) and management practices (crop rotation, irrigation, chemical control etc.) may prevent diseases and reduce losses in agriculture. However, the most appropriate strategy for plant diseases controlling consists of developing resistant varieties and selecting for high seed quality (Maloy 2005). One of the typical features of modern agriculture is to increase productivity through external chemical inputs, including fertilizers, pesticides, fungicides and herbicides (Abd-Alla et al. 2014). However, the excessive chemical application may cause environmental disorders that could affect both soil quality and plants' health. In addition, chemical application promotes resistant pathogen emergence and decreases beneficial organism populations in soil (Silva et al. 2004).

Common bean (*Phaseolus vulgaris* L.) is one of the most important legumes worldwide because of its extensive production, higher consumption and nutrient values, and its commercial impact (Popovic et al. 2012). It provides (15%) of protein, (30%) of the caloric requirement of the world's population (McConnell et al. 2010). The annual common bean production is about 17 million tons, constituting an important agricultural crop and a protein source for human diets (Karakaya and Özcan 2001). However, bean production is confronted with numerous attacks of devastating diseases. Among them, common blight is one of the five major bacterial diseases of bean crops, leading to important yield losses (Broughton et al. 2003; Silué et al. 2010). The disease can attack leaves, stems, pods, seeds and is difficult to deal, where chemical control by copper compound formulations seems inefficient (Fourie 2002; Zanatta et al. 2007).

The necessity to reduce chemical products for more sustainable agriculture obliged researchers and farmers to seek for new solutions. Among these, using beneficial bacteria for soil health restoration and plant growth, improvement seems to be promising (Vale et al. 2010). Thus, bacteria like those belonging to the genera *Pseudomonas*, *Azospirillum*, *Bacillus*, *Azotobacter*, *Burkholderia*, and *Enterobacter* are able to colonize plant rhizosphere, root and shoot interior/surface, and can promote plant growth. Such bacteria are referred to as Plant Growth Promoting Rhizobacteria (PGPR) (Glick and Bashan 1997; Beneduzi et al. 2012; Gupta and Kaushal 2017). The genus *Pseudomonas* belongs to Gamma-Proteobacteria. They are Gram negative, motile and mostly oxidase positive. The presence of a characteristic green fluorescent pigment subdivides the genus *Pseudomonas* to fluorescent and non-fluorescent Pseudomonads. Many studies had shown that Pseudomonads are potential biological control agents (Wei et al. 1996). They are largely used to protect a wide range of plants from several biotic attacks, enhancing plant growth and effectively reducing many plants diseases' severity (Beneduzi et al. 2012).

The overall objective of this work was to isolate soil bacteria belonging to the genus *Pseudomonas* from different regions in western Algeria. After that, the isolated bacteria were biochemically identified, screened for their BOX-PCR profiles, investigated for their ability to produce PGP traits such as HCN, Indole Acetic Acid, siderophores, phosphate solubilization and their antibacterial activity against the isolated common blight-causing *Xanthomonas axonopodis*pv. *Phaseoli*

var. *fuscans* (Xapf). Finally, the effectiveness of two selected *Pseudomonas* isolates in protecting bean seedlings against the aforementioned pathogen and their role as promoters of bean germination was evaluated.

Material and methods

Soil sampling and bacterial isolation

Rhizospheric and non-rhizospheric soil samples were collected between 2010 and 2014 from the provinces of Mascara, Saida, Sidi Bel Abbes, Relizane and Tlemcen, all located in western Algeria (Table 1). Samples were collected in sterile bags and immediately transported to the laboratory for bacterial isolation using the method described by Pepper and Gerba (2004). Thus, 10 g of each sample were mixed in 90 mL PBS (Phosphate Buffered Saline) and placed under agitation (200 rpm/30 min). One mL of the obtained solution was inoculated in nutrient broth then incubated for 48 h (28 °C). 100 µL of the resulting cultures were spreaded on Petri dishes containing either Cetrimide (Harmonized, Hemedia®) or 10% egg white-supplemented King B agar (BD Difco™) (Garibaldi 1967) and incubated at 28 °C or 37 °C/48 h. Characteristic colonies of the genus *Pseudomonas* were purified and conserved for further studies. Phytopathogenic isolate named Xapf and 50 isolates were selected from 10 different sites and named from P1 to P50 as shown in Table 1.

Identification and characterization of isolates

Biochemical and physiological characterization

The production of characteristic pigments of the genus *Pseudomonas* (pyoverdine and pyocianine) was realized following the protocol of Cho and Tiedjen (2000). Determination of lecithinase production, gelatin liquefaction, KOH solubility, oxidase tests, and utilization of L-arabinose, D-xylose, sodium tartrate, glucose, D-alanine, L-tryptophan, sorbitol and L-arginine as sole carbon source was tested as described by Goszczynska et al. (2000). Strain abilities to grow at 4 °C and 41 °C were determined according to Rhodes (1959). Nitrate reduction and denitrification activities were tested as described by Delif et al. (2005). Pectinase production, Polyhydroxybutirate (PHB) accumulation, and isolates' pathogenicity on bean leaves were tested according to Reetha et al. (2014), Panigrahi and Badveli (2013) and Saettler et al. (1989), respectively. Following the obtained results, species identification was established according to the dichotomous keys and LOPAT test described by Holt et al. (1994) and Lelliott et al. (1966), respectively. Differences between the obtained Biovars were determined as described by Palleroni (1984). Species and Biovars identification was achieved using the Advanced Bacterial Identification Software (ABIS) available on http://www.tgw1916.net/bacteria_Pseud.html.

The phytopathogenic isolate was identified using specific phenotypic characters of colonies obtained on

Table 1 Soil sampling, geographic localization and bacterial isolation

Site of sampling	Geographic localization	Number and code of the isolated bacteria	Plant/rhizosphere
Tighennif (Mascara)	35°24′ N 0°19′ E	Xapf	*Phaseolus vulgaris* L
Tizi (Mascara)	35°18′N 0°04′E	Strains from P1 to P9	*Phaseolus vulgaris* L
Tizi (Mascara)	35°18′N 0°04′E	Strains from P10 to P15	*Allium cepa* L
Rocade Nord (Sidi Bel Abbes)	35°13′N 0°37′W	Strains from P16 to P21	Balk soil
Ain El Hadjar (Saida)	34°45′N 0°09′E	Strains P22 and P23	Vicia sativa
Slatna (Mascara)	35°24′N 0°11′E	Strains from P24 to P27	Vicia sativa
Abou Tachefine (Tlemcen)	34°54′N 1°19′W	Strain P28	Balk soil
Bendaoud (Relizane)	35°42′N 0°31′E	Strains from P29 to P35	Pisum sativum
Ain Fares (Mascara)	35°27′N 0°14′E	Strains from P36 to P40	Solanum tuberosum
Bou Hanifia (Mascara)	35°18′N 0°02′W	Strains from P41 to P45	*Avena sativa* L
Mohammadia (Mascara)	35°35′N 0°03′E	Strains from P46 to P50	*Hordeum vulgare* L

the semi-selective medium PTSCA. The isolate's identity was then confirmed by pathogenicity test on bean leaves.

BOX-PCR fingerprinting

DNA extraction was performed following William et al. (2012). Pure colonies of the isolated bacteria were treated by standard protocol for PCR manipulation. After DNA extraction, BOX-PCR were performed using mixtures of 1 × PCR buffer, 2 mM MgCl2,0.1 Mm dNTPs, 0.8 μM BOX-A1R primer (5′-CTA CGG CAA GGC GAC GCT GAC G-3′), 5% dimethylsulfoxyde, 1.3 U TaqDNA polymerase and standardized 15 ng of genomic DNA in a final volume of 30 μL. The PCR mixtures were denatured (94 °C/1 min), subjected to 35 cycles (94 °C/1 min, 45 °C/1 min and 72 °C/2 min) and to a final extension (72 °C/10 min). PCR products were checked on 1.5% agarose gel by electrophoresis (100 V/30 min) and revealed using Gel DOC (Biorad).

PGP traits characterization

Phosphate solubilization

Bacteria were streaked on Pikovskaya's agar medium containing (per liter of distilled water): (0.5 g yeast extract, 10 g dextrose, 5 g $Ca_3(PO_4)_2$, 0.5 g $(NH4)_2SO_4$, 0.2 g KCl, 0.1 g $MgSO_47H_2O$, 0.0001 g $MnSO_4H_2O$, 0.0001 g $FeSO_47H_2O$ and 15 g agar, pH 7.4). After incubation at 28 °C/6 day (s), isolates inducing a clear zone around their colonies were considered as Phosphate Solubilizing Bacteria (PSB). Results were expressed as the clear halo diameter: PSI = A-B, where A is the total diameter (Colony + halo zone) and B is the colony diameter (Nautiyal 1999).

Siderophores production

Siderophores were detected on Chrome Azurol-S (CAS) agar medium. Isolates were streaked on Petri plates containing CAS medium and incubated at 30 °C for 48 h. Formation of orange halos around colonies indicated siderophore production by bacteria (Yeole et al. 2001). In addition, siderophores were quantified as described by Christina et al. (2015). Accordingly, Isolates were grown in modified King's B broth and incubated under continuous agitation (28 °C/24–30 h/150 rpm). After incubation, cell free supernatant, obtained by centrifugation (14.000×*g*/10 min at 4 °C), was mixed with 0.5 mL CAS solution. The obtained color was spectrophotometrically (630 nm) recorded after 20 min incubation in darkness. A control, prepared with 0.5 mL CAS solution and 0.5 mL uninoculated King's B broth, was used. Siderophore percentage was estimated through color variation as compared to the control using the formula: [(Ar - As) /Ar] × 100, where (Ar) is the control absorbance and (As) is the sample absorbance (CAS assay solution + Cell-free supernatant).

Hydrogen cyanide secretion

Hydrogen cyanide (HCN) production was screened as described by Lorck (1948). Bacterial isolates were streaked on nutrient agar supplemented with 0.44% L-glycine. Whatman paper discs were soaked in an alkaline picrate solution (0.5% picric acid, 2% Na_2CO_3) and deposited in the lids of Petri dishes. The system was sealed with parafilm and incubated at 28 °C for 4 day(s).

To quantify HCN amount, the method described by Bakker and Schippers (1987) was performed. Thus, King's B broth-containing tubes, amended with 0.44% filter sterilized L-glycine, were inoculated with bacterial isolate. Whatman paper strips (10 cm × 1 cm) were soaked in an alkaline picrate solution and introduced at the tubes tops. Tubes were then sealed with parafilm and incubated at 28 °C/72 h. Color changes from yellow to brown or reddish brown indicates moderate or high HCN production, respectively. The total cyanide content (TCC) in Whatman paper was estimated by using the following equation: TCC (ppm) = 396 × OD (510 nm), where OD is the broth absorbance after incubation.

Indole acetic acid synthesis

Isolates were evaluated for their ability to synthesize IAA, largely known to stimulate plant growth. Therefore, Qualitative IAA estimation was determined as described by Bric et al. (1991). Luria Bertani (LB) medium supplemented with 5 mM L-tryptophan was used. IAA and/or its analogs production in the medium lead to a red-pink color

formation. For other indoles, color becomes yellow or brown yellow.

Quantitative IAA evaluation was realized following the protocol of Jeyanthi and Ganesh (2013). 10 mL of medium were inoculated with fresh bacterial cultures then incubated under shaking for 72 h (28 °C/120 rpm). Then, 1 mL of supernatant, obtained by centrifugation (2.253×*g*/20 min at 4 °C), was mixed with 3 mL Salkowski's reagent (50 mL of 35% of Perchloric acid, 1 mL of 0.5 M FeCl3). After incubation (room temperature in darkness), IAA amount was spectrophotometrically determined (540 nm). Finally, IAA concentration was estimated by the standard curve prepared using pure IAA solution (25–200 μg/mL).

Isolation of *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans*

To isolate the common blight-causing *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans* (Xapf), a Modified method of Saettler et al. (1989) was used. Bacteria was isolated from4 to 5 months old of infected *Phaseolus vulgaris L* plant showing common blight characteristic symptoms from Tighennif (Mascara) (Table 1). Therefore, infected leaves were macerated in sterile demineralized water and serially diluted (up to 10^{-2}) in sterile saline water. 100 μL of each dilution were spread on Petri plates containing PTSCA medium and incubated at 28 °C for 3 to 6 days. Typical colonies of Xapf were then purified and tested to confirm their pathogenicity on bean plants of trifoliate leaf stage (2 to 3 weeks after sowing). Infection was performed by soaking a sterile gauze in the pathogenic bacterial suspension (standardized to 10^8 CFU/mL using distilled water). The first two trifoliate leaves were weakly rubbed by the gauze on both sides and plants were maintained at 25 ± 5 °C under high humidity for 5 to 7 day (s).

In vitro antibacterial activity of the isolated bacteria against Xapf

Isolate antibacterial activity was tested following a modified protocol of Skathivel and Gnanamanickam (1987). A standardized Xapf culture suspension was prepared by scraping 48–72 h-old culture. The suspension was then molted with a previously cooled (42 °C) King's B agar and versed in Petri plates. From 7 day (s) old cultures, the antagonistic isolates were inoculated by deposing agar disks of (6 mm) on the plates already inoculated with the pathogen. Controls without antagonist (sterile agar disks) and without pathogen (sterile king's B agar) were used. The plates were then incubated at 28 °C and periodically checked to detect inhibition zones appearance around the antagonist agar disks during 2 to 3 days. The results of the antibacterial activity were expressed as measuring three times the clear halo diameter: Aa = D-d, where D is the total diameter (Colony + halo zone) and d is colony diameter. Based on this test, two strains (P7 and P25) were selected and tested for their ability to inhibit the common blight and to enhance been germination.

Biocontrol of bean common blight caused by the isolated Xapf

The isolated bacteria that revealed in vitro antibacterial activities were in vivo screened for their ability to biocontrol the common bean blight caused by our isolated pathogen Xapf (Al-Saleh 2014). Therefore, 48 h-old cultures of two selected bacteria (P7 and P25) were centrifuged (14.000×*g* for 10 min) and bacterial pellets were washed twice with sterilized distilled water. The strains' optical densitywas adjusted to ($OD_{610\ nm} = 0.45$) to obtain bacterial suspensions of about 10^7 CFU/mL. The phytopathogen Xapf was grown on King's B agar medium and incubated at 28 °C for 48–72 h. Pure Xapf colonies were scraped from the cultures, re-suspended in sterile distilled water, and their optical density was spectrophotometrically adjusted to ($OD_{610\ nm} = 0.6$), corresponding to 10^8 CFU/mL.

Four treatments were realized to study the possible role of the two selected in vitro antagonistic isolates and their combination [P (7) + P (25)] proportion (1:1) on Xapf propagation on bean leaves. Each treatment was composed of 10 plants and prepared by spraying bean leaves with the prepared bacterial suspensions. Three days later, plants were infected by spraying the Xapf suspension on their leaves until complete saturation of the leaves surface. Controls without treatment or with only infected with the isolate Xapf were used. Inoculated plants were immediately enclosed in plastic bags at high humidity for 48 h.

At the end of the experiment, disease severity was estimated by measuring the percentage of infected leaves. In addition, symptoms intensity was determined using a predefined scale (from F0 to F3): F0

(without task), F1 (one unbroken task with oily aspect), F2 (1 to 3 brown tasks surrounded with a halo or leaf side-necrosis), F3 (50% of the leaf with pale or yellow necrosis with diffuse color) (Bernier 2011).

Effect of the bacterial isolates on bean seed germination

The two bacterial isolates, previously assayed as biocontrol agents of common bean blight, were tested, individually and in combination, for their effect on bean seed germination using a modified protocol of Shweta et al. (2008). Seeds were disinfected with 2% sodium hypochlorite solution for 3 min then washed three times with sterile distilled water. Seeds of each treatment were soaked in a standardized bacterial suspension (10^7 CFU/mL) for 3 h, dried and deposited in agar water-containing Petri plates (0.8% agar). Controls with seeds soaked in sterile distilled water were similarly prepared. Three Petri dishes by treatment and 10 seeds in each of them were used. After incubation at 25 °C for 5 days in darkness, germinated seeds (G) and the number of lateral roots (NLR) were counted. A numerical method was used to measure primary root lengths (PRL) and root area (RA) using the Image J software.

The stimulation percentage of each parameter (S %) as compared to the control was expressed using the formula: $S\ \% = [(test^{mv}\text{-}control^{mv})/control^{mv})] \times 100$; where ($control^{mv}$) is the mean value of the control for each parameter, and $test^{mv}$ is the mean value of the treated seeds for the same parameter.

Statistical analysis

In vivo study of seed germination assays was analyzed by one-way ANOVA. Seed growth properties and biological control of common blight were analyzed by two-way ANOVA. All results were statistically compared to their corresponding controls using Graph Pad Prism V. 6.0.The DendroUPGMA (available on: http://genomes.urv.cat/UPGMA/) was used to realize dendrograms of carbon sources assimilation and BOX-PCR profiling of the isolated bacteria. For this, the pattern was score specified with binary characters (1 = positive growth and 0 = absence of growth) for carbon sources assimilation and (1 = presence and 0 = absence of band) for BOX-PCR profiling (Garcia-Vallve et al. 1999).

Results

Identification and characterization of the isolated bacteria

Biochemical and physiological identification of 50 bacterial isolates (supplementary material) had shown high metabolic diversity. Thirty-four isolates developed pale green pigmentation in King's B agar medium and liberated a characteristic odor of soft mist. Exposing of Petri plates to UV light under (365 nm) revealed a fluorescent green pigment. Two isolates produced pyorubin (red pigment) which is a specific pigment of the species *Pseudomonas aeruginosa*. That indicated that bacteria forming a total of 36 (72%) isolates belong to the fluorescent Pseudomonas group; whereas, 14 (28%) bacterial isolates do not produce this specific characteristic pigment, indicating that these isolates belong to the non-fluorescents Pseudomonas group. Within 15 days incubation, 49 (98%) isolates had shown positive reaction for oxidase, 37 (74%) isolates for reduction of nitrates and 50 (100%) isolates solubilize KOH. They were able to assimilate most of carbon sources tested: 45 (90%) isolates assimilated glucose, 50 (100%) isolates assimilated alanine, 20 (40%) isolates assimilated arginine and 27 (54%) isolates assimilated xylose. 15 (30%) isolates assimilated L-tryptophan, 31 (62%) isolates assimilated sodium tartrate and 29 (58%) isolates liquefied gelatin. 2 (4%) isolates only accumulated PHB. A total of 19 (38%) isolates grew at 4 °C and 11 (22%) isolates grew at 41 °C.

Biochemical and physiological characterization

Identification of bacterial isolates by ABIS software revealed that 36 (72%) isolated bacteria belonged to fluorescent Pseudomonads, whereas the non-fluorescent Pseudomonads were represented by 14 (28%) isolates. The predominance of *Pseudomonas fluorescens* Biovars (1, 2, 3, 4 and 5) 23 (44%) was clearly observed. Among them, *Pseudomonas fluorescens* Biovars 5 was represented with 10 isolates (20%). Moreover, *Pseudomonas putida* Biovars (A) and *Pseudomonas cichorii* were represented by four isolates for each one. The less represented species principally belong the non-fluorescent Pseudomonads: *P. stutzeri* 3 (6%), *P. pseudomallei* 2 (4%), *P. plantarii* 2 (4%),

P. cepatia 2 (4%) isolates, *P. corrugota* 1 (2%), *P. lini* 1 (2%) and *P. maltophila* 1 (2%) isolate.

The distribution of the dominant isolated bacteria *P. fluorescens* Biovar 5 according to their samples showed their abundance in *Pisum sativum* (Bendaoud) and *Hordium vulgare* L (Mohamadia) (Table 2).

Numerical analysis of phenotypic characteristics revealed high polymorphism among *Pseudomonas* isolates. The 50 isolates tested are grouped into five major phenons (Fig. 1). Phenons 1, 2, 3, 4, and 5 that consist a total of 2 (4%), 9 (18%), 11 (22%), 3 (6%) and 25 (50%) isolates, respectively. Phenon 5 was the main and diversifies, assembling fluorescent and non-fluorescent Pseudomonads species, including isolates belonging to *P. putida* Biovar A. The second major phenons 2 and 3 regrouping heterogeneous *Pseudomonas* species. *P. fluorescens* Biovar 5 isolates were distributed between these two clusters. Phenons 1 and 4 were the less represented phenons, comprising only isolates belonging to *Pseudomonas alcaligenes* and *Pseudomonas stutzeri,* respectively.

Table 2 Results of biochemical identification, obtained using the Bacterial Identification Software (ABIS)

Isolate	Species	Isolate	Species
P1	*P. pseudomallei*	P26	*P. grimontii*
P2	*P. plantarii*	P27	*P. grimontii*
P3	*P. corrugota*	P28	*P. cichorii*
P4	*P. alcaligenes*	P29	*P. fluorescens* Biovar 5
P5	*P. stutzeri*	P30	*P. fluorescens* Biovar 5
P6	*P. plantarii*	P31	*P. fluorescens* Biovar 5
P7	*P. cepatia*	P32	*P. alcaligenes*
P8	*P. pseudomallei*	P33	*P. fluorescens* Biovar 1
P9	*P. cepatia*	P34	*P. fluorescens* Biovar 2
P10	*P. fluorescens* Biovar 2	P35	*P. fluorescens* Biovar 5
P11	*P. fluorescens* Biovar 2	P36	*P. fluorescens* Biovar 5
P12	*P. stutzeri*	P37	*P. fluorescens* Biovar 5
P13	*P. fluorescens* Biovar 2	P38	*P. fluorescens* Biovar 3
P14	*P. fluorescens* Biovar 3	P39	*P. fluorescens* Biovar 3
P15	*P. aeruginosa*	P40	*P. putida* Biovar A
P16	*P. fluorescens* Biovar 2	P41	*P. fluorescens* Biovar 2
P17	*P. stutzeri*	P42	*P. aeruginosa*
P18	*P. cichorii*	P43	*P. putida* Biovar A
P19	*P. lini*	P44	*P. putida* Biovar A
P20	*P. fluorescens* Biovar 2	P45	*P. cichorii*
P21	*P. cichorii*	P46	*P. fluorescens* Biovar 5
P22	*P. maltophila*	P47	*P. fluorescens* Biovar 3
P23	*P. fluorescens* Biovar 4	P48	*P. fluorescens* Biovar 5
P24	*P. putida* Biovar A	P49	*P. fluorescens* Biovar 5
P25	*P. grimontii*	P50	*P. fluorescens* Biovar 5

BOX-PCR fingerprinting

Molecular diversity of *Pseudomonas* isolates was also confirmed by the molecular level. BOX-PCR showed 29 haplotypes characterized by 1 to 7 bands, ranging from 100 bp to 1600 bp size. Numerical BOX-PCR analysis differentiated the species belonging to *Pseudomonas*. DNA fingerprinting pattern result in a dendrogram showing five different clusters (Fig. 2). Cluster V formed the major cluster, comprising 21 (42%) isolates belonging to fluorescent and non-fluorescent Pseudomonads groups, including most isolates belonging to *P. putida* Biovar A. Clusters I and II were the second major cluster, comprising 5 (10%) and 6 (12%) isolates respectively. Those two clusters grouped the largest part of *Pseudomonas fluorescens* Biovar 5 isolates. Clusters III and IV were the less represented clusters including 1 (2%) and 2 (4%) isolates, respectively. 14 (28%) isolates did not fall into the dendrogram and revealed together their distinct profile and genotypic identities.

BOX-PCR profiling results analysis indicate better classification of the isolates taxonomically and simultaneously with the carbon source assimilation. Comparison between BOX-PCR profiling and metabolic groups suggested this observation is valid. Clear resemblances among phenons and clusters were observed.

PGP trait characterization

Functional properties of the isolated bacteria showing qualitative and/or quantitative production of secondary metabolites were regrouped in (Table 3).

Phosphate solubilization

33 (66%) *Pseudomonas* isolates induced solubilization of tri-calcium phosphate on Pikovskaya's medium by forming clear zones around the colonies. Whereas, 22 (34%) isolates did not show any halo around their colonies, indicating that they do not

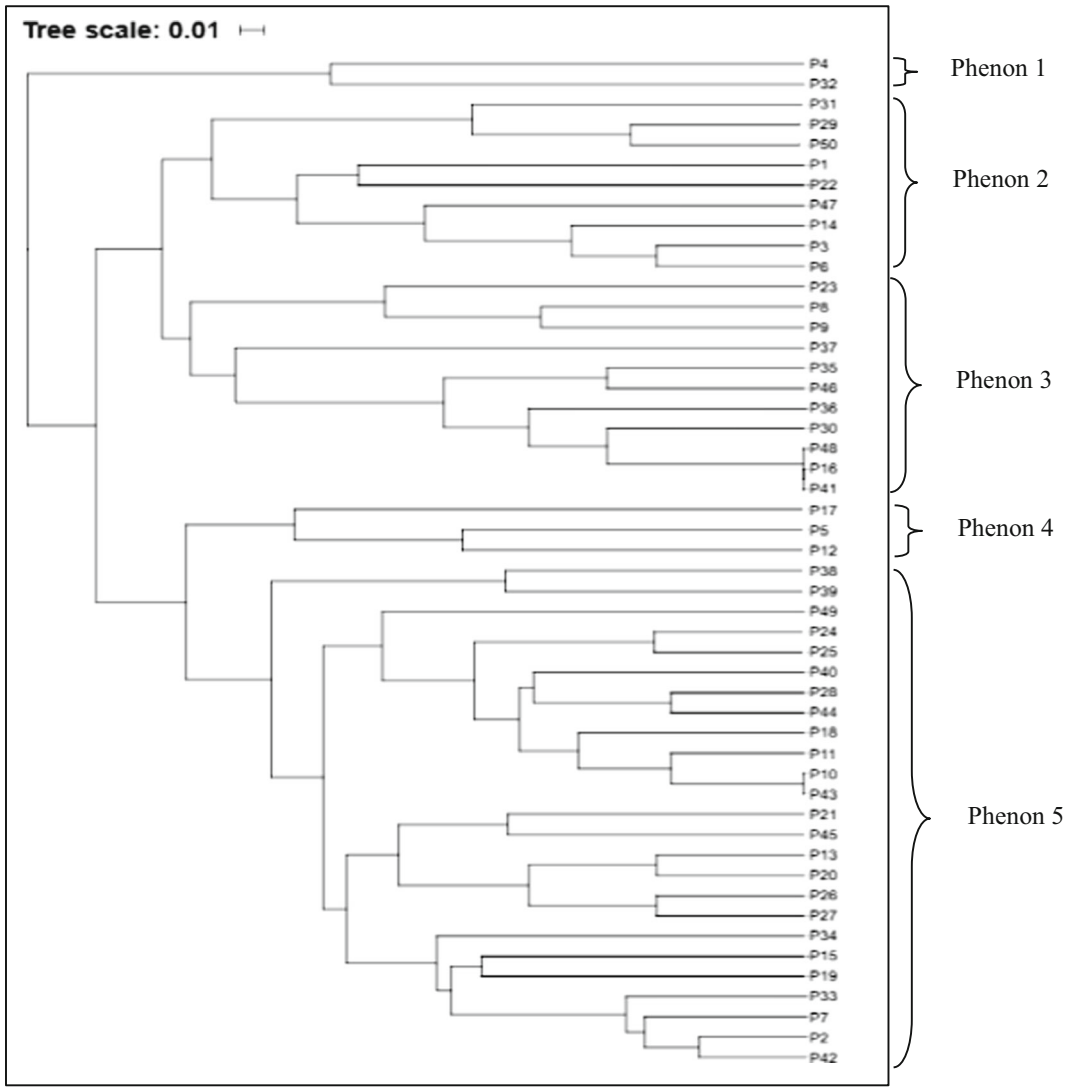

Fig. 1 Dendrogram obtained by comparing physiological and biochemical characteristics of the 50 isolated bacteria

solubilize tricalcium phosphates. Phosphate solubilization index (PSI) ranged from 0.5 to 10.5 after 6 days inoculation. Isolate P17 induced the highest PSI (10.5); moderate PSI was registered for P44 and P45 (4.5), while P20 and P21 gave a PSI of about 6 and 6.5, respectively. The weakest PSI was recorded for isolates P1, P10, P12, P24 and P32 (0.5). Other isolates induced PSI index varying from 1 to 3 or did not solubilize phosphate.

Siderophores production

After 72 h of incubation, siderophore production by 28 (56%) *Pseudomonas* isolates on CAS agar medium showed the appearance of clear zones with orange color, representing iron chelation. 22 (34%) isolates do not show any change surround growth. Quantitative analysis using CAS solution revealed that all isolates produced siderophores**.** Percentages of siderophores production varied from $3.79 \pm 2.66\%$ to $52.12 \pm 3.35\%$ for isolates P5 and P31, respectively.

Hydrogen cyanide secretion

Evaluation of the capacity of isolates to produce hydrogen cyanide revealed a qualitative change of Whatman paper N°1 color from yellow to reddish brown that

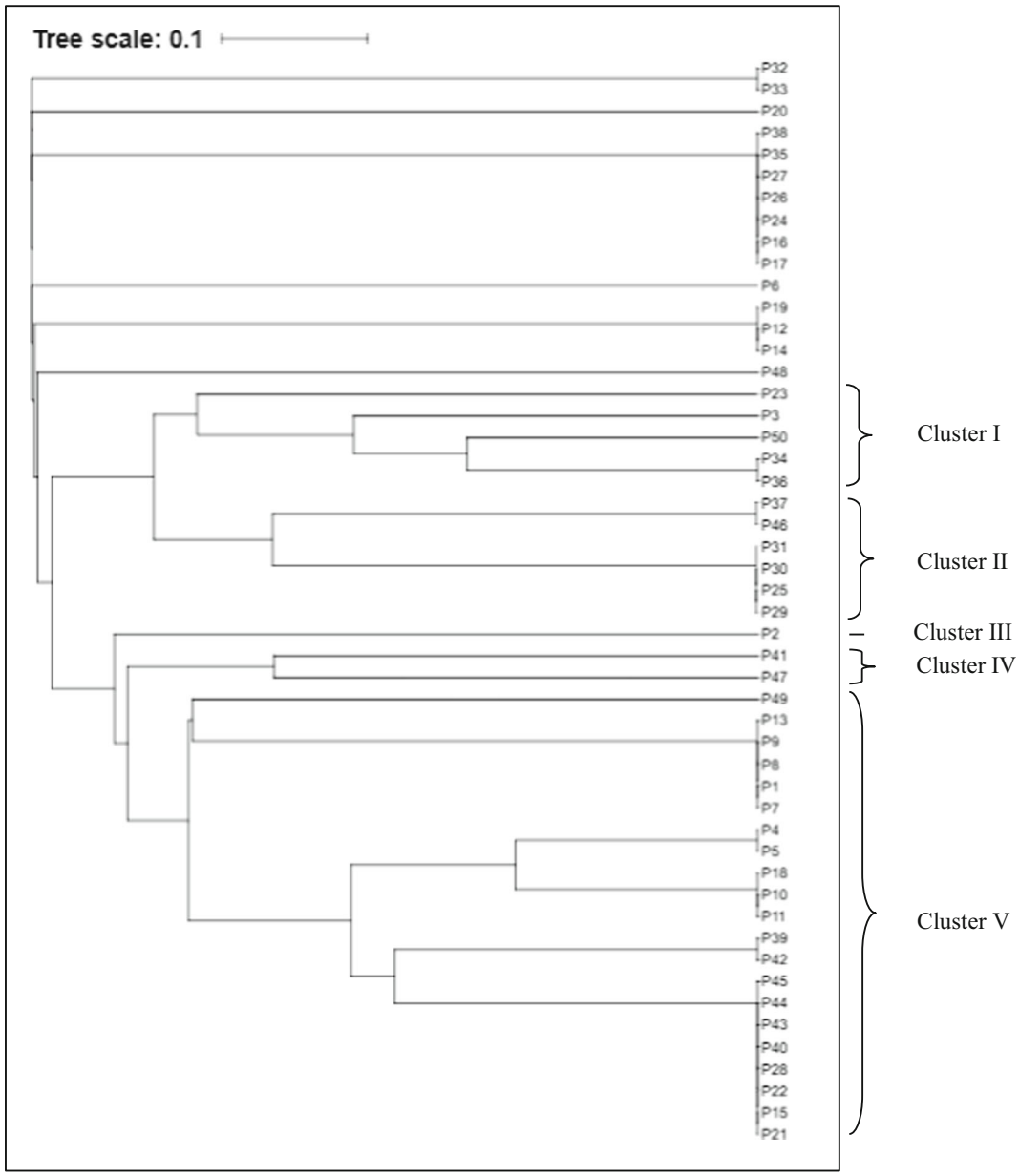

Fig. 2 Dendrogram obtained by comparing BOX-PCR results obtained with the 50 isolates

confirmed production of HCN. Of all isolates tested, 24 (48%) had shown HCN production on nutrient agar and 26 (52%) shown negative production. Quantitative HCN production in King's B broth revealed only 12 (24%) of isolates producers and 38 (76%) shown a negative result. Isolate P45 had exhibited the lowest production of 20.59 ± 2.86 ppm, whereas the highest production was unregistered for the isolate P1 of 83.95 ± 1.81 ppm. Other isolates had shown moderate or none HCN production.

Indole acetic acid synthesis

Qualitative IAA production revealed that 28 (56%) isolates produced IAA. 22 (43%) isolates shown negative results. Quantitative IAA production on TSB broth supplemented with (0.5%) of L-tryptophan revealed 21 (42%) isolates IAA producers; whereas, 29 (48%) isolates did not produce IAA. A varying level of IAA production was recorded for the low production of 3.62 ± 0.90 μg/ml for isolate P34, to the high production

Table 3 PGP traits: qualitative and quantitative estimation

Isolate	HCN (ppm)		PSI	SED (%)		IAA (μg/mL)	Aa (mm)
P1	83.95 ± 1.81	+++	0.5	22.58 ± 0.69	++	95.88 ± 0.41	6.67 ± 0.58
P2	0	–	0	33.18 ± 0.45	++	119.85 ± 32.1	4.00 ± 0
P3	0	–	0	15.91 ± 4.04	++	0	4.33 ± 0.58
P4	0	+	0	22.42 ± 3.09	–	99.04 ± 0.20	0
P5	26.80 ± 1.14	++	0	3.79 ± 2.66	+	0	0
P6	0	–	1	9.39 ± 3.19	++	0	0
P7	0	+	0	4.39 ± 1.89	++	73.69 ± 0.20	24 ± 0
P8	43.69 ± 3.37	++	0	20.00 ± 2.76	++	105.00 ± 2.34	0
P9	0	–	0	7.88 ± 1.89	++	0	0
P10	0	–	0.5	36.21 ± 1.84	–	0	0
P11	0	–	1	29.24 ± 2.50	–	0	0
P12	0	–	0.5	35.30 ± 2.92	–	141.42 ± 0.35	0
P13	0	+	1.5	35.15 ± 0.95	–	133.33 ± 8.16	0
P14	0	+	1	43.64 ± 0.95	++	0	5.33 ± 0.58
P15	27.85 ± 3.08	++	1	40.15 ± 0.26	+	0	0
P16	0	–	2	36.67 ± 2.33	++	0	7.33 ± 0.58
P17	39.73 ± 2.39	+	10.5	38.48 ± 1.14	–	152.14 ± 0.61	0
P18	0	–	0	38.64 ± 1.20	–	0	0
P19	0	–	3	31.97 ± 1.84	–	0	0
P20	0	–	6	39.55 ± 1.20	–	0	5.33 ± 1.15
P21	0	–	6.5	36.82 ± 1.20	+	0	2.33 ± 0.58
P22	37.49 ± 8.5	+	0	39.85 ± 2.10	+	118.57 ± 1.07	3.33 ± 0.58
P23	0	–	1.5	9.85 ± 1.84	–	0	5.33 ± 0.58
P24	0	–	0.5	43.03 ± 0.26	–	0	0
P25	0	–	0	48.03 ± 0.95	–	132.61 ± 2.03	26.67 ± 2.31
P26	61.12 ± 0.91	+++	0	51.06 ± 0.95	–	0	6.67 ± 0.58
P27	0	–	1.5	51.52 ± 2.74	–	0	5.67 ± 0.58
P28	0	–	0	45.76 ± 2.66	+	69.57 ± 2.58	0
P29	61.51 ± 0.46	+	0	38.33 ± 1.46	++	0	0
P30	0	+	2	45.30 ± 1.39	+	0	0
P31	0	+	0	52.12 ± 3.35	++	0	0
P32	0	–	0.5	43.18 ± 0.45	–	0	0
P33	0	–	0	40.30 ± 0.95	–	0	7.67 ± 0.58
P34	0	–	2.5	45.45 ± 2.76	+	3.62 ± 0.90	7.33 ± 1.15
P35	0	–	1	34.39 ± 0.95	+	37.19 ± 0.55	7.33 ± 1.15
P36	68.11 ± 4.12	+++	1	19.70 ± 3.87	+	93.14 ± 0.36	10.67 ± 1.15
P37	0	+	2	47.27 ± 3.87	++	0	0
P38	0	+	2	26.06 ± 0.95	+	25.40 ± 0.41	6.67 ± 0.58
P39	0	–	1.5	9.70 ± 7.94	–	13.98 ± 0.55	0
P40	0	–	0	36.06 ± 1.05	–	61.48 ± 1.76	0
P41	0	+	0	36.67 ± 1.39	–	8.50 ± 0.36	0
P42	0	+	1	25.76 ± 1.05	–	148.98 ± 1.44	2.00 ± 0
P43	0	–	2.5	17.73 ± 4.04	+	0	0
P44	0	+	4.5	29.24 ± 0.69	+	28.02 ± 1.03	10 ± 0

Table 3 (continued)

Isolate	HCN (ppm)		PSI	SED (%)		IAA (μg/mL)	Aa (mm)
P45	20.59 ± 2.86	+	4.5	10.61 ± 1.14	–	0	10.00/±0
P46	0	+	3	23.33 ± 2.15	+++	16.71 ± 1.99	12.67 ± 1.15
P47	54.52 ± 2.29	++	1	40.00 ± 2.08	+	0	10.00 ± 1.73
P48	0	–	3	23.48 ± 1.84	+++	0	0
P49	0	–	1	13.79 ± 8.68	–	0	0
P50	63.49 ± 3.41	+++	1	3.94 ± 1.39	+	0	0

Qualitative and/or quantitative determination of PGP traits PSI: phosphate solubilization index. SED: Siderophores. Aa: Antibacterial activity. **(+++)** Highly positive reaction, **(++)** Moderate positive reaction, **(+)** Low positive reaction, **(−)** Negative reaction

of 152.14 ± 0.61 μg/ml for isolate P17 and 148 ± 1.44 μg/ml for isolate P42.

Isolation of *Xanthomonas axonopodis* pv.*phaseoli* var. *fuscans*

Phytopathogenic isolate Xapf had shown characteristic colonies of *Xanthomonas axonopodis* pv. *Phaseoli* on the semi-selective medium PTSCA (Fig. 3). Colonies were yellow, round, mucoid with whole margins. In addition, the fuscans type was distinguished by diffusion of brown pigment, after incubation at 28 ± 2 °C for 3 to 6 days. Confirmation of Xapf identification was obtained by verification of pathogenicity on bean leaves; in the initial stage of the disease (Fig. 4), symptoms infection on leaves appears as a small lesion soaked with water. When the disease progresses (Fig. 4), lesions become larger and were developed to brown, dry tasks with yellow halos. These lesions consist of irregular regions of dry brown tissue, frequently produced at the margins of leaves.

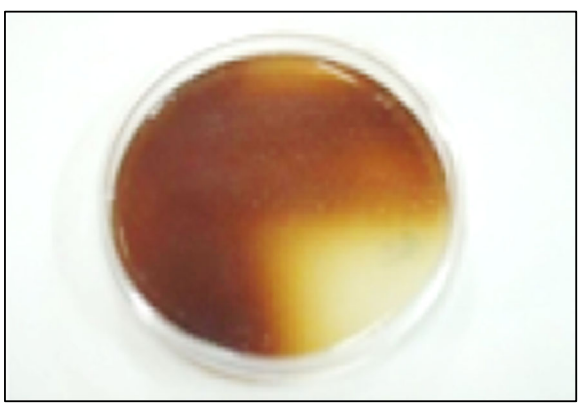

Fig. 3 Characteristic brown diffusible pigment of *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans* isolates on PTSCA medium

In vitro antibacterial activity of the isolated bacteria against Xapf

Results obtained in the present study by the dual culture technique revealed that 23 (46%) of *Pseudomonas* isolates exert potent antibacterial activity against *Xanthomonas axonopdis* pv. *phaseoli* var. *fuscans* (Table 3). The highest antibacterial activities recorded were 26.67 ± 2.31 mm for *P. grimontii* P25, followed by 24.00 ± 0 mm for *P. cepatia* P7.The lowest antagonistic activity was registered for P42 *Pseudomonas aeruginosa* of 2.00 ± 0 mm.

Biocontrol of bean common blight caused by the isolated Xapf

The two antagonistic isolates *P. grimontii* P25 and *P. cepatia* P7 were tested for their biological control potential against common blight been caused by the pathogenic isolate Xapf (Fig. 5). Application of isolates P7 and P25 alone or in combination reduced the number of common blight tasks on bean leaves as compared to the control leaves only infected by the pathogenic isolate.

Biological control results analyzed by ANOVA indicated that application of the two isolates P7 or P25 alone or in combination decreased the disease intensity (Fig. 6). Significant decrease of symptom scales F0 (leaf without task) and F3 (50% of the leaf with pale or yellow necrosis with diffuse color) were observed, whereas symptom scales F1 (one unbroken task with oily aspect) and F2 (1 to 3 brown tasks surrounded with a halo or leaf side-necrosis) were not highly affected. The highest increase of non-infected leaves, represented by scale F0, was obtained through inoculation by the

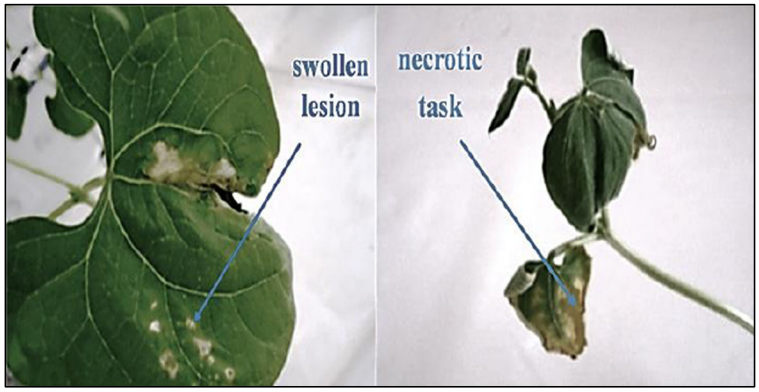

Fig. 4 Pathogenicity test of *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans* (Xapf) showing swollen lesion as initial stage of common blight bean (on the left) and necrotic task as advanced stage of common blight bean (on the right)

isolate P25 (71.82%) followed by co-inoculation P7 + P25 (73.6%) as compared to the control (57.2%). The three inoculations decreased diseased intensity scale F3. Highest decrease was recorded through inoculation by isolate P7 up to (9.8%) compared to the control (33%); followed by the isolate P25 and co-inoculation with P7+ P25 (11.3 and 11.94%, respectively).

Effect of the isolated bacteria on bean seed's germination

Inoculation of *Phaseolus vulgaris* seed with *Pseudomonas* isolates *P. cepatia* P7, *P. grimontii* P25, and co-inoculation with P7 + P25 increased seed germination as compared to uninoculated controls (Fig. 7). Application of isolate P7 alone was highly

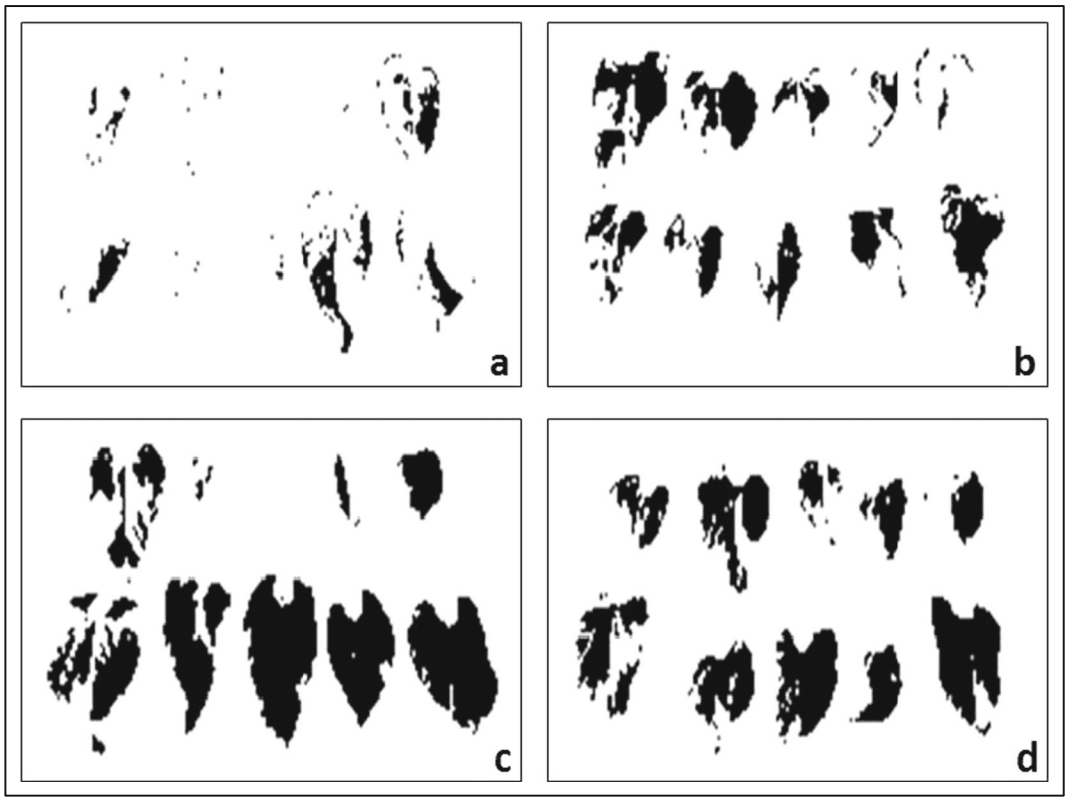

Fig. 5 High contrast-uncolored leaves pictured from the experiment "biological control of common blight caused by Xapf". **a**: leaves only infected by the pathogen Xapf (control). **b**: leaves infected by the pathogen Xapf and treated by the combination P7 + P25. **c**: leaves infected by the pathogen Xapf and treated by P25. **d**: leaves infected by the pathogen Xapf and treated by P7. Black regions of leaves indicate unaffected zones

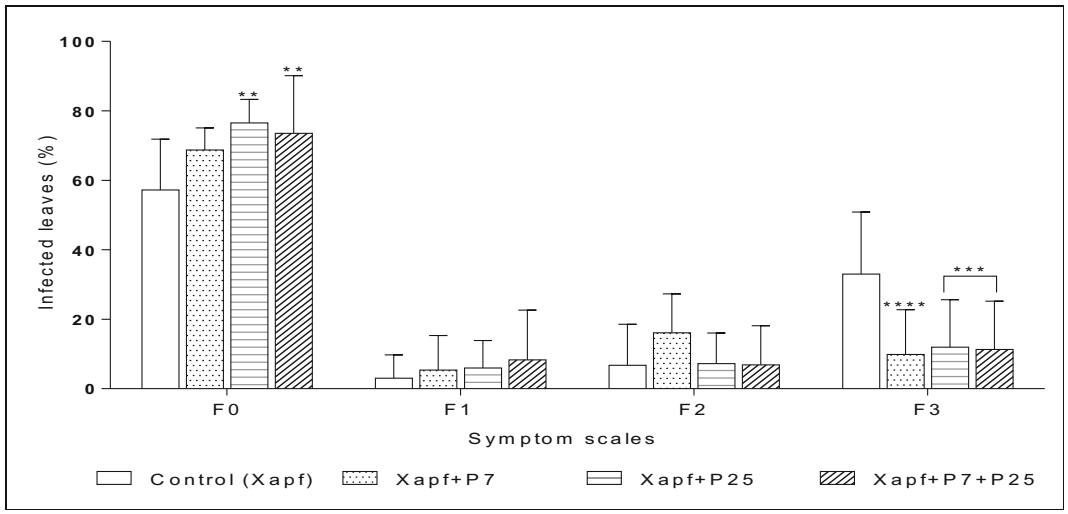

Fig. 6 Biological control of common blight intensity caused by Xapf by *Pseudomonas*. Values represent the percentages of infected leaves. F0, F1, F2 and F3 represent symptom scales as shown in material and methods (**: significant difference at $p < 0.01$; ***; significant difference at $p < 0.005$; ****: significant difference at $p < 0.001$)

effective on seed germination. A significant increase of seed germination when treated with isolate P7 was recorded (66.6%). However, P25, alone and in combination with P7, did not show any significant increase in seeds germination rate.

Results obtained from seedling growth properties treated by *Pseudomonas* isolates, applied alone or in combination, shown a significant increase of SNLR (Stimulation of Number of Lateral Root) and SPRL (Stimulation of Primary Root Length). Root areas SRA (Stimulation of Root Area) was not highly enhanced (Fig. 8). Application of isolate P7 increased (SNLR) up to 232% and co-inoculation P7 + P25 up to 223%. Inoculation of seed by P7 increase (SPRL) up to 48.5%. Thus, P7 was highly effective on stimulation of seed growth properties. Followed by the co-inoculation P7 + P25 that revealed considerable stimulation of (SNLR).

Discussion

In the last 20 years, bacteria of the genus *Pseudomonas* have occupied an important place among the most studied soil bacteria. Their remarkable capacity to inhibit several phytopathogenic agents in soil and to enhance

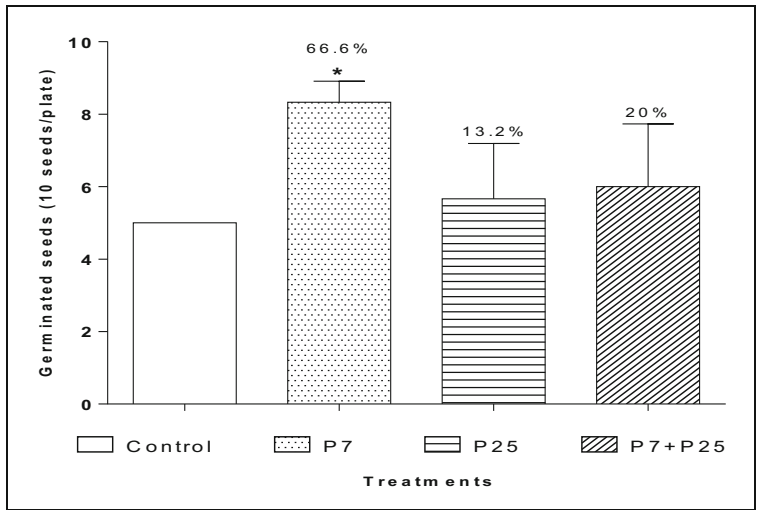

Fig. 7 Effect of *Pseudomonas* isolates on seed germination of *Phaseolus vulgaris* L. Values represent the average number of germinated seeds. Percentages represent comparisons to control. (*: significative difference at $p < 0.05$)

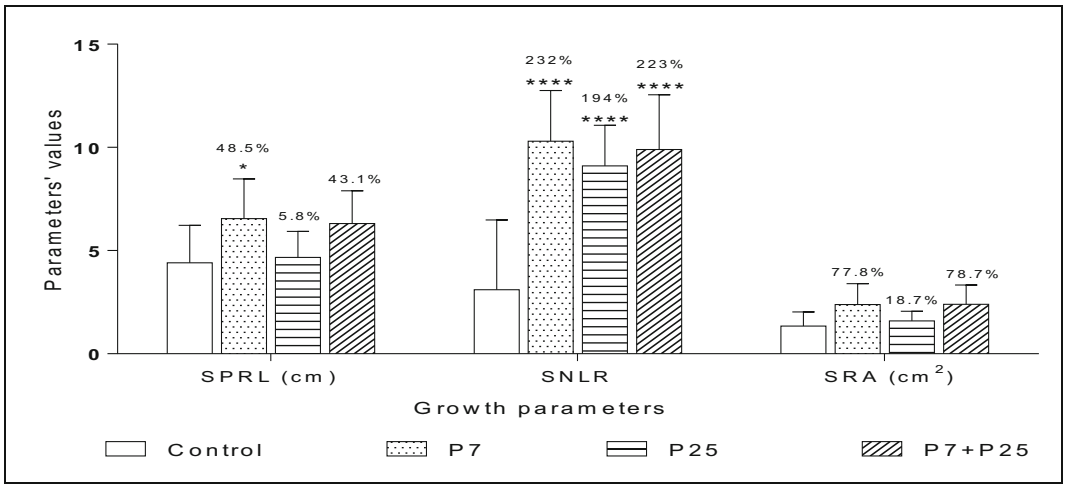

Fig. 8 Effect of *Pseudomonas* isolates on seed growth properties of *Phaseolus vulgaris* L. Values represent the stimulation of the properties of germinated seeds. The percentages represent the stimulation of seed properties compared to the control (100%). SPRL: Stimulation of primary root length. SNLR: Stimulation of number of lateral roots. SRA: stimulation of root area (*: significant difference at $p < 0.05$; ****: significant difference at $p < 0.0001$)

plant growth is mainly due to their high metabolic diversity (Wei et al. 1996).

In this work *Pseudomonas fluorescens* Biovar (1, 2, 3, 4 and 5) was the most isolated species, representing 22 (44%). Biovar 5 represented the most dominant Biovar with 20% isolates, followed by *P. putida* Biovar A and *P. cichorii* 4 with 8% isolates for each. This was probably due to the medium selectivity (cetrimide) and the incubation temperature (from 30 °C to 37 °C). We also revealed the abundance of *P. fluorescens* Biovar 5 in *Pisum sativum* and *Hordeum vulgare* rhizospheric soil samples. Similar results were reported by Meliani (2012), mentioning that among 70 strains, the dominating *Pseudomonas* bacteria from divers rhizospheric plant were *P. fluorescens* and *P. putida*, representing 38.57% of the total. Sutra et al. (2000) also reported that among 58 fluorescent *Pseudomonas* isolates, 41 were identified as *P. fluorescens* Biovar 5 and 17 as *P. putida* Biovar A in banana rhizospheric soil.

The capacity of *Pseudomonas* to use different carbon sources is an important characteristic in microorganism natural selection. The characterization results of our isolates showed their ability to use most of the tested carbon sources, which is consistent with the results reported by Palleroni (1993). The same author revealed the high metabolic and ecological diversity of the *Pseudomonas* genus. Otherwise, Bossis et al. (2000) confirmed that Levan production, denitrification, gelatin liquefaction and L-tryptophan assimilation are characteristic of different Biovars of *P. putida* and *P. fluorescens*.

Pseudomonas capacity to assimilate several carbon sources can affect and contribute to their diversity in a specific environment. According to Zak et al. (1994), the rhizobacterial functional diversity is defined as the degradation rate that determines the whole comportment of a microbial community against several substrates assemblage. The *Pseudomonas* diversity observed in our results (five physicochemical phenons) may occur from their extracellular enzymes' diversity and their high adaptability from one rhizospheric soil to another. As the phylogenic diversity that was investigated by BOX-PCR profiling showing five distinct clusters forming from the lowest (2%) to the highest (47.62%) of the isolated species. BOX-PCR uses repetitive extragenic palindromic-PCR (BOX A1R). This single primer binds to repetitive sequences in bacterial genomes that are conserved and specific for each species (Fitriyah et al. 2013). In this study, 14 (28%) isolates did not fall into any of the clusters, indicating the high *Pseudomonas* variability and diversity in western Algeria.

Physicobiochemical and phylogenic diversity of *Pseudomonas* isolates is probably due to the characteristics of soil samples and the types of plant rhizospheres from which they were isolated. Plant root exudates could be largely implicated in determining the soil microbial diversity and metabolic/functional diversity of rhizospheric bacteria (Lakshmi et al. 2015). In addition, the environmental characteristics, differing from one sampling site to another, are also

determining factors of the obtained bacterial diversity (Fierer and Jackson 2006). Our results confirm that the genus *Pseudomonas* is able to colonize the rhizospheres of several plants, being highly competent in environments with different biological and physicochemical characteristics (Wei et al. 1996).

The main interest in investigations with different species of the genus *Pseudomonas*, other than their high diversity and rhizo-competence, lies in their ability to produce several metabolites of high agricultural interest such as IAA, siderophores, hydrolytic enzymes and antibiotic compounds (Dorjey et al. 2017). In this work, 33 (66%) isolates showed high ability to solubilize insoluble phosphate with a PSI ranging from 0.5 to 10.5. Several reports indicated that different bacterial species, particularly rhizosphere colonizing bacteria, have the ability to liberate organic phosphates or to solubilize insoluble inorganic phosphate compounds. These bacteria make available the soluble phosphates to plants and, in return, gain root-borne carbon compounds, mainly sugars and organic acids, necessary for bacterial growth (Khan et al. 2010). In addition, all isolates tested in this study produced siderophores. Siderophores are low molecular weight metabolites that play an important role in supplying iron to plants (Radzki et al. 2013). Bacteria of the genus *Pseudomonas* are known to produce high amounts of siderophores such as pyochelin, pseudobactin and pyoverdine (Neilands 1989; Mezaache et al. 2014). Such characteristics are largely used to select beneficial soil bacteria that help to supply iron to plant, but also to compete with other pathogenic organisms for the limited available-iron in soil. Siderophores production by bacteria is a key mechanism by which they eliminate pathogenic fungi by competition, and hence promoting plant growth and defenses (Peer et al. 1990). Moreover, siderophores are important for their producers survival, colonization and iron metabolism control (Adams et al. 1994).

Twenty-four percent of the isolated *Pseudomonas* showed ability to produce HCN. It is a secondary metabolite, commonly produced by rhizosphere Pseudomonads. HCN may directly enhance plant growth or inhibit plant pathogens development (Schippers et al. 1990). 24% of the isolated bacteria produced HCN. HCN production depends the substrate amino acids composition. Thus, glycine is the direct precursor of microbial cyanide production (Askeland and Morrison 1983). The rate of microbial HCN production in the rhizosphere also depends on the amino acid composition of root exudates. In addition, environmental factors such as light intensity and soil water potential may also affect root exudation rate, exudate composition, and hence HCN production (Schippers et al. 1990). HCN can indirectly interfere with phosphorus availability and, when synthesized by a PGPB, it appears to act synergistically with other biocontrol traits employed by the same bacterium (Hinsinger 2001; Olanrewaju et al. 2017). Moreover, HCN is known as an inhibitor of the cytochrome c oxidase as well as other important microbial metallo-enzymes, participating in pathogen biocontrol and plant growth enhancement (Nandi et al. 2017).

IAA-producing PGPR are believed to increase root growth and root length (Vessey 2003). In this study, 21% isolates produced IAA. Isolates P7 and P25 produced 73.69 ± 0.20 and 132.61 ± 2.03 μg/mL IAA, respectively. Resulting in a significant increase of bean SNLR and SPRL. IAA production controls a wide variety of processes in plant development and plant growth: low concentrations of IAA can stimulate primary root elongation; whereas high IAA levels stimulate the formation of lateral roots, decrease the primary root length, and increase root hair formation (Vacheron et al. 2013).

The high in vitro antibacterial activities of the two isolates *P. grimontii* P25 and *P. cepatia* P7 could be explained by their ability to secrete one or a combination of different PGP compounds (Dorjey et al. 2017). Qualitative and quantitative characterization of secondary metabolites such as phosphate solubilization, siderophores, HCN and IAA are the main characteristics in PGPR selection. Such metabolites can contribute to Xapf biological control, but also to bean growth stimulation observed in this work. Chin-A-Woeng et al. (2003) reported that the metabolic diversity of PGPR makes of them interesting candidates as biofertilizers and biological control agents of plant diseases. In addition, their high ability to colonize roots and to maintain strong density and diversity population is probably due to their rhizocompetence and their capacity to metabolize efficiently several root exudates compounds.

This study of biological control of Xapf by isolated bacteria revealed that isolate *P. cepatia* P7, reduced significantly the disease intensity, then isolate *P. grimontii* P25 or co-inoculation with P7 + P25, although isolate P25 applied in vitro gave the highest antibacterial activity. *P. cepatia* strain was used as a potentially effective biological control agent by Cartwright and Benson (1995). Bacterial biocontrol agents

(BCA) effective in vitro are not necessarily effective in vivo. Giorgio et al. (2016) reported that out of 162 Rhizobacteria isolates, 60 inhibited the growth of common bacterial blight (CBB) in in vitro condition. But only six of these, when applied to seeds, reduced disease symptoms in in vitro and greenhouse assays**.** Corrêa et al. (2017) suggest also a relationship between the region of origin of Xapf strains and the efficacy of BCA to control the commune blight bean.

In this study, *P. cepatia* P7 alone induced the highest significant seed germination rate. Also, seed growth analysis revealed that the isolate P7 was the most effective on seed growth stimulation (SNLR and SPRL). This bacterium was isolated from rhizospheric soil of *Phaseolus vulgaris*, which presupposes that the isolation origin could play an important role in seed germination, bacterial colonization and adaptation. The co-inoculation with P7 + P25 was less effective, probably due to competition that maintained a lower density population of isolate P7. Lemanceau (1992) reported that some bacterial strains, belonging in particular to the group of Pseudomonas fluorescent spp., seem to improve the germination of seeds of different plants (colza, corn and tomato). Kapilan and Thavaranjit (2015) reported also that the application of *Pseudomonas* strains showed high promoting effect on bean seed germination. Boruah et al. (2003) reported that bacterization bean seed with fluorescent Pseudomonas strains significantly increased the root biomass and length of roots. Bacterial strains produced more than one-fold increase in root length that ranged from 13 to 17%. The inoculation of bean with bacterial strains *P. extremorientalis* TSAU20 and *P. chlororaphis* TSAU13 increased root length, even under saline stress conditions (Egamberdieva 2011). Kragelund and Nybroe (1996) mentioned that there was competition between *Pseudomonas fluorescens* Ag1 and *Alcaligenes eutrophus* JMP134 in co-inoculation (pJP4) during colonization of barley roots.

Variation in the degree of promoting effect on seed germination depends on the variety of plant seeds, bacterial species used, the rate of colonization, amount of substance causing the effect, and the internal physical factors of seeds (Carrillo-Castañeda et al. 2002; Krishnaswamy and Seshu 1990). The ability of a strain or bioinoculant combination to promote plant growth changes with the cultivated variety. This could be due to the variation in the rhizosphere dynamics of a given bacterial isolate which in turn could be caused by the change in plant root exudates (Kumar et al. 2015). In terms of this in vivo study, we can conclude that application of isolate *P. cepatia* P7 was more effective on seed germination, seed growth properties, and biological control of Xapf compared to *P. grimontii* P25 or their concomitant application. Isolate P7 was isolated from rhizospheric soil of *Phaseolus vulgaris*, when inoculated on the same plant, it showed high effectiveness as biological agent and biofertilizer. It shows the crucial role of origin isolation in biological control of plant disease and growth stimulation, besides the traits properties of PGPR.

Conclusion

In conclusion, the selected *Pseudomonas* isolates could play a crucial role in common bean blight biocontrol and beans yield promotion through siderophore, HCN and IAA production, phosphate solubilization, and other metabolite secretions. The selected strains also showed high stimulation of bean seed germination. Further studies are needed to investigate their security and their long-term effect on plants, soil and the microbial community of the inoculated environment. Finally, these bacteria could present promising agricultural applications in the future.

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