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Isolation, Screening, Characterization of PGPR of Lentils *Lens culinaris*

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ABSTRACT

In plant growth-promoting rhizobacteria (PGPR) are soil bacteria that colonize plant roots and improve their growth by different ways. The main aim of the present study was the isolation and the characterization of the rhizospheric bacterial strains belonging to the genus of Bacillus, which were responsible for the growth of Lens culinaris. For this purpose, five samples of soils were collected from several region of Sidi Bel Abbès located, located in North West of Algeria such as Ain Témouchent, Tessala, ITCMI, Sfisef and Kaid Belarbi, which were investigated for their capability of promoting plant growth of Lens culinaris. Twenty four bacterial strains belonging the of the genus of Bacillus were isolated and initially selected by the study of germination process in vitro by the determination of some parameters such as root length, stem length, dry weight and fresh grain weight like the B8, B11, B14 and B15. Furthermore, the isolated, selected promoting growth bacterial strains were subsequently characterized by the study of indol-2-acetic acid (IAA) production, phosphate solubilization, nitrogen assimilation and screened for their antifungal activity against Fusarium oxysporum. The obtained results indicated that the isolated, selected bacterial strains (B15, B10) has manifested a excellent antagonistic activity against Fusarium oxysporum, with a maximal diameter of inhibition zone 50, 50 mm respectively. Whereas, the both isolates (B5, B6) have showed a average, with a maximal diameter of inhibition zone 25, 25 mm respectively. Moreover, the isolated, selected bacterial strains (B4, B7, B12) has indicated the lowest antagonistic activity against Fusarium oxysporum, with a diameter of inhibition zone of 10 mm. Furthermore, the both selected bacterial (B4, B15) were further characterized by the phosphorus solubilization with the varied index between 2.8-3,6.

Furthermore, the results revealed that the isolated strains presented similar characteristics to the genus of Bacillus sp, where both isolated, selected bacterial strains B7 and B15 has presented an excellent effect for promotion germination of Lens culinaris

Keywords: PGPR, *Bacillus* sp, *Lens culinaris*, IAA, Germination.

INTRODUCTION

Food legumes account for a important share of food and feed, which are rich in proteins and provide a significant amount of fixed nitrogen useful for cereals, reducing production costs and limiting groundwater pollution by nitrates in fertilizers [1-3]. In Algeria, the cultivated lentils (*Lens culinaris*) area increased from 26,000 ha in 1969 to 1,500 ha in 1997, with very low fluctuated yields [4,5]. The pathogenic Fungi were the main limited development lentil factors, which were caused wilting or root rot diseases [6]. Plant growth-promoting rhizobacteria (PGPR) are soil bacteria, which were colonized plant roots and improved their growth by several mechanisms such as phytohormones, siderophore production and solubilization of phosphate, inhibition of pathogenic microorganisms and detoxification of the environment [7-13]. Furthermore, among the PGPR cluster, the genus of *Bacillus* was the most potential genera due to their ability to form spores and their increased adaptation to commercial formulation and field application [14].

Bacillus species are Gram-positive, aerobic, facultative anaerobic, which were characterized by the presence of catalase, endospore-forming, chemoheterotrophic rod-shaped bacteria, usually motile with peritrichous flagella [15].

Members of the *Bacillus* genus found generally in soil represented a wide range of physiological abilities, which allowed their bacterial growth in several environmental conditions and competed other undesirable organisms, due to their capability to form extremely resistant spores and to produce metabolites with antagonistic activity against pathogenic microorganisms [16]. Due the diversity of Plant growth-promoting rhizobacteria (PGPR), capacity for colonization, mechanisms of action, formulation, their application for the management of agricultural systems should facilitate their development as reliable components.

The main aim of the present work was the isolation of *Bacillus* sp from the cultivated rhizospheric soil with lens (*lens culinaris*) from several region of North West Algeria such as Ain Témouchent, Tessala, ITCMI, Sfisef and Kaid Belarbi, the the screening of the their capacity for production of numerous secondary metabolites such as hydrocyanic acid production HCN, inorganic phosphates solubilization and the proteins hydrolysis and their identification has been achieved.

MATERIALS AND METHODS

Collection and preparation of soil samples

50 g of 5 rhizospheric soil samples were collected from several region located in North West Algeria such as Ain Témouchent, Tessala, ITCMI, Sfisef and Kaid Belarbi. Furthermore, the samples were placed in sterile plastic bags and transported immediately in refrigeration at temperature of 4°C to the laboratory for further analysis.

Isolation of Bacillus species

Primary isolation of *Bacillus* has been achieved by weighting of 10 gr of the collected rhizosphere soil from several sites and their taking into a 250 ml conical flask, where a volume of 90 ml of sterile distilled water was added, heated at temperature of 80°C for 10 min. The prepared serial dilution by the using of the nutrient culture medium was inoculated with a volume of 1 ml of the overnight culture, homogenized on the vortex for 2 min. Furthermore, a volume 0.1 ml of the bacterial suspension was spread on the plates of nutrient agar medium, incubated at temperature of 37 ºC for 24 hours. The obtained colonies were selected, picked and surface-streaked several times until purification. The purified isolated bacterial strains were stored in 10% glycerol at -80° C for further analysis.

Biochemical characterization of rhizobacteria

The isolated bacterial strains was inoculated on the nutrient agar plates, incubated at temperature of 37ºC for a period of 3 days and their morphological characteristics has been studied by the determination shape, size, elevation, appearance of the surface, margin, color, odor, and the produced pigmentation.. Moreover, the Gram coloration, spore-forming bacilli and the motility were further investigated.

Characterization of Bacillus isolates for PGP traits

Nitrogen hydrolyse

The fixation of molecular nitrogen was investigated on a solid free-nitrogen culture medium. The isolated, selected PGPR was inoculated by streaking on the N-free culture medium incubated at temperature of 30°C for a period of 48 hours. The presence of bacterial growth indicated their capability of nitrogen fixing.

Production of indol-3-acetic acid

The exploration of the indol-**3**-acetic acid production was investigated by the inoculation of the 20 bacterial isolates on the peptone broth culture medium, enriched with tryptophan (100µg/ml), as a precursor of auxin, which were an important plant hormone and the incubation at room temperature for a period of 48 hours. The culture supernatant was harvested by centrifugation at 6000 rpm for 10 min. The concentration of the produced indol-**3**-acetic acid was estimated by the mixing of a volume of 2 ml of culture supernatant with 2 drops of orthophosphoric acid and a volume of 4 ml of the followed Salkowski reagent (50 ml, 35% perchloric acid; 1 ml 0.5 $FeCl_3$), according to the described method by Rahman [17]. Furthermore, the mixture was agitated on the vortex, incubated at room temperature for 25 min and the lecture has been achieved by the apparition of pink color production and the concentration was determined by the measure of the optical density was performed at 530 nm by the using aspectrophotometer. Indole-3-acetic acid was used as standard for determination of the produced quantity of IAA, expressed as µg/ml of culture filtrate [12].

Phosphate solubilization

The phosphate was the most commonly limiting nutrient required for the growth of plants. Many microorganisms are able to solubilize the present unavailable forms of phosphate in the soil. For this purpose, the isolated bacterial strains was inoculated by streaking on the solid culture medium of Pikovskya's, incubated at temperature of 28°C for a period of 7 days. The lecture was performed by the examination of the grown bacterial colonies and the data was recorded. The phosphate solubilization was

estimated by the apparition of clear zone around the inoculated colony on the solid culture medium, which was containing insoluble mineral phosphate (tricalcium phosphate or hydroxyapatite) as sole source of phosphore.

Antifungal activity

The antagonistic activity of the isolated, selected bacterial strains belonging to the genus *Bacillus* against pathogenic fungi *Fusarium oxysporum* was investigated by the using of the modified dual culture method on Potato Dextrose Agar (PDA) medium [13]. For this purpose, the pathogenic fungi *Fusarium oxysporum f. sp. ciceris* furnished by INRAA (Algeria) was inoculated on the culture medium at the center of the Petri plats and the isolated, selected bacterial strains *Bacillus* were streaked on the solid PDA culture medium, which was left 3 cm from the margin. Plates without antagonist isolated, selected bacterial strains *Bacillus* were served as control. The plates were incubated at room temperature for 4-7 days and the antagonistic activity of the isolated, selected bacterial strains *Bacillus* was evaluated by measurement of the percent inhibition of mycelial growth of pathogenic fungi *Fusarium oxysporum* in the direction of actively growing bacteria according to the described formula by Kumar et al. [14], where is:

$$1-(a/b) \times 100\%$$

a: Distance between fungi in the center of Petri dish to test isolate,

b: Distance between fungi in the center of Petri dish to blank are without *Bacillus* isolate).

Seed germination assay

The isolated, selected *Bacillus* was investigated for their ability to promote seedling growth by the using of the slight modified described methods by Dey [19]. For this purpose, the surface of seed germination was sterilized by the using of sodium hypochlorite solution for 5 min and washed with sterile distilled water for 5 times and the isolated, selected *Bacillus* was cultivated on the nutrient agar culture medium plates, incubated at room temperature for 24 hours. Furthermore, a nine pre-germinated sterilized seed was soaked in a volume of 0.1 ml of bacterial broth cultures, which was contained approximately 10^9 cells/ml, incubated at room temperature for 30 min. The germination parameters were investigated by the determination of the length, the primary root, shoot, and numbers of lateral roots after 7 days incubation times. The obtained results was analyzed by the use of the statistically analysis of variance (STATITCF).

RESULTS

Soil analysis and bacterial isolation

The primary analysis of samples indicated that the used soil has alkaline pH-value with low proportion of lime and good proportion of organic matter and presenting a silty-sandy texture. The physical and chemical characteristics of soil samples are illustrated on the Table 1. Furthermore, the obtained results of physical and chemical characteristics of the analyzed soil samples indicated the clay -loam area nature of Ain Témouchent soil and the silt-loam area nature of Tessalah and Sfisef soil respectively. Whereas, the analyzed soil samples of both regions such Kaid Belarbi, ITCMI manifested the Sandy-loam, clay-silty nature respectively. The clay content was considerably increased in the investigated soil samples and has reached a maximum of 42% in the analyzed soil samples of Tessalah. Due their cation exchange capacity (CEC), the contained a large amount of clay, humus in soil samples may induce a buffered effect of pH change. The results obtained indicated that the analyzed soil samples has manifested alkaline pH value, where were varied 8-8.98 and contained a high content of organic matter of 2-4%, which promoted

any e microbial activity. Furthermore, the measure of electrical conductivity of the analyzed soil samples was range between 0-0.5 ms, which indicated unsalted soils sample.

Biochemical characteristics of Bacillus species

The macroscopic observation and the study of the morphological and physiological characteristics of the twenty four bacterial strains isolated from several region of Sidi Bel Abbès such as Sfisef, Kaid Belarbi, ITCMI, technical institute of market and agricultural cultures, Tessalah, presented a positive reaction for catalase, oxydase and the presence deforming central endospore, which was explained by their membership to the genus of *Bacillus* sp. A further micro-morphological examination of the isolated selected bacterial strains indicated the presence bacillary form and Gram-positive stains, which was contained a varied size between 2-10 µm in long and 0.5-2 µm in large with irregular edges and round or truncated ends (Table 2). Moreover, 10 of the isolated, selected bacterial strains were motile, where eight bacterial strains have manifested a positive reaction by the presence of catalase (Table 2). Furthermore, twenty four isolated bacterial strains belonging to the membership of *Bacillus* were initially investigated for germination process *in vitro* by the determination of some parameters such as root length, stem length, dry weight and fresh grain weight of the selected bacterial strains (B8, B11, B14 and B15).

Table 1: Determination of the physical and chemical parameters of soil samples

Soil samples	EC	pH	C	Organic matter	Total limestone	Active limestone	Sand	Silt	Clay
Kaid Belarbi	0. 52	8.98	2.03	4.06	09%	2.4%	62.03	24.55	13.42
Tessala	0.34	6.47	0.69	1.38	43%	12%	28.97	42.55	42.46
Sfisef	0.34	8.31	1.61	3.22	09%	06%	36.34	40.37	30.66
Ain. Temouchent	0.48	8.04	3.56	6.16	38%	10%	30	55	15
Itcmi	0.30	8.02	1	2	40%	12%	22.11	39.10	40

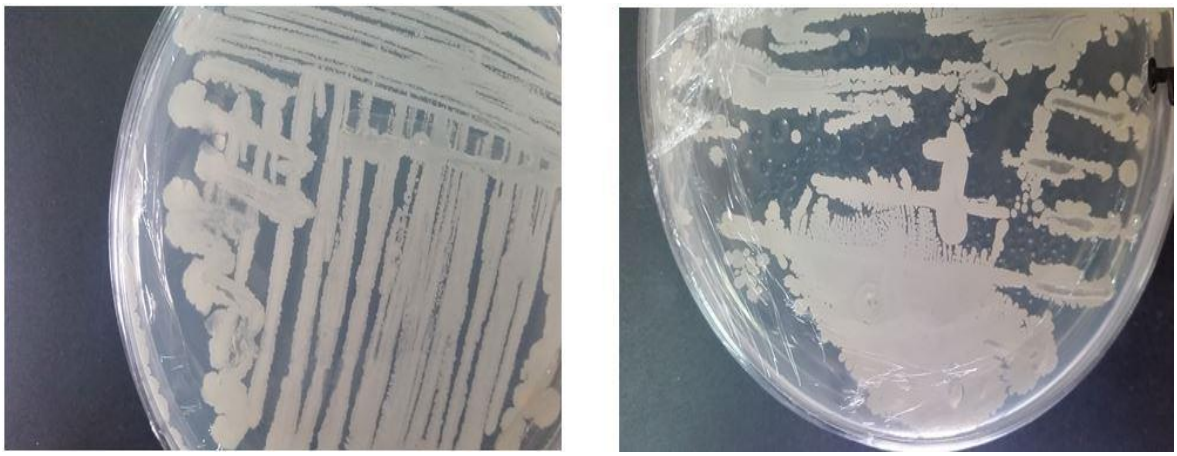

Figure 1: Macroscopic observation of the isolated, selected phosphate solublization isolate B2 belonging to the genus of *Bacillus*, inoculated on the solid culture medium of Pikovskya's, incubated at temperature of 28°C for 7 days.

Table 2: Illustration of the Biochemical characteristics of the isolates strains belonging to the genus of *Bacillus* spp, inoculated on the solid culture medium of Pikovskya's, incubated at temperature of 28°C for 7 days.

Tests Isolates	Gram stain	Spore	shape	motility	Oxydase	Catalase	Respiratory type
B1	-	+	Spherical	-	-	+	aerobic
B2	+	+	jagged ends	-	-	+	aerobic
B3	+	+	Rode	+	-	+	aerobic
B4	+	+	rode	+	-	+	aerobic
B5	+	-	rode	+	-	-	aerobic
B6	+	+	rode	-	+	+	aerobic
B7	-	-	rode	+	+	+	aerobic
B8	+	+	rode	+	+	+	aerobic
B9	+	-	rode	+	-	+	aerobic
B10	+	+	rode	-	+	+	aerobic
B11	-	+	rode	+	+	+	aerobic
B12	+	-	rode	+	+	+	aerobic
B13	+	+	rode	+	+	-	aerobic
B14	+	-	rode	-	-	+	aerobic
B15	+	-	rode	+	+	+	aerobic
B16	+	+	rode	+	-	-	aerobic
B17	+	-	rode	+	+	+	aerobic
B18	+	+	rode	+	+	-	aerobic
B19	+	+	rode	+	+	+	Aerobic
B20	+	-	rode	+	-	+	Aerobic
Note: (+) Positive reaction; (-) Negative reaction							

Morphological characteristics of Bacillus isolates

Plant growth promoting activities

The isolated, selected bacterial strains belonging to the genus of *Bacillus* were screened *in vitro* for their plant promoting of *Lens culinaris* activities. The obtained results of plant growth promoting (PGPR) were illustrated in Table 3. Furthermore, the determination of some properties beneficial effects of the isolated, selected bacterial strains on plants promoting by the provision of nutrients or indirectly by the protection against plant pathogens has been investigated.

Seed germination assay

The exploration of the effect of the inoculation of the isolated, selected phosphate solubilization bacterial isolates on the seed germination of lentil has been investigated. The obtained results indicated that from the investigated, selected phosphate solubilization bacterial isolates, both (B8 and B16) has considerably affected the development of the lentil weight of shoots and root length. Furthermore, the study of seed germination percent, shoot length, root length and seedling vigor. varying effects in growth promoting of lentil seeds**.** Some isolates showed a very pronounced PGPR effect on sprouted seed weight improvement as well as longitudinal root growth. The isolates B11 and B4, B13 and B14 can be mentioned. The lowest response has been observed with the isolate B20 (Figures 1-4).

Figure 2: Effect of the inoculated,isolated, selected phosphate solubilization isolates (B8 and B16), on the seed germination seedlings inoculation on germination.

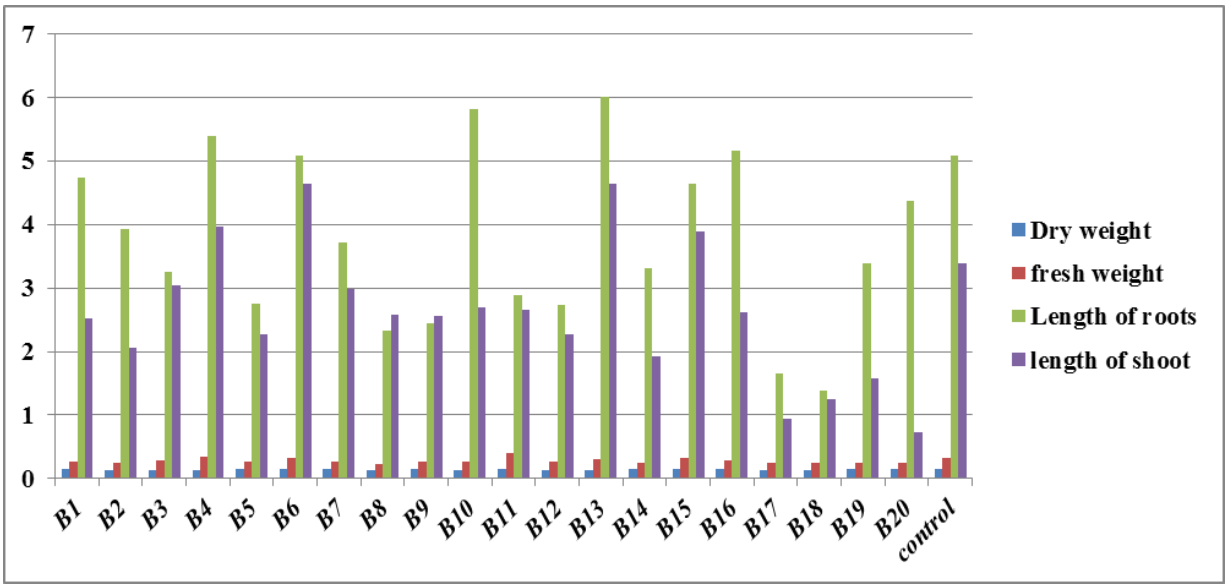

Figure 3: Effect of the inoculation of the isolated, selected phosphate solubilization isolates on the development of dry and fresh weight and shoots and root length lentil.

Evaluation of plant growth promotion potential

The inoculation of *Lens culinaris* seeds with the 20 isolated strains showed an important increasing of plant growth parameters after 7 days of sowing. The increase weight of lentil weight and the development shoot and root length due to the treatment of *Lens culinaris* culture with bacterial isolates (B11, B4) was ranged between 0.13-0.40 g and 0.12-0.34 g respectively. Similarly, bacterial isolates (B13, B10) induced a considerable increase of the length of shoots (5.9 cm and 5.8 cm) and root (4.5 cm and 2.5 cm) by the inoculated of *Lens culinaris* respectively. Where, a maximum root length was observed by the bacterial strain B13 (4.5 cm) followed by strains B10 (2.5 cm) compared to control. Furthermore, a maximum shoots length was observed by the bacterial strain B13 (5.9) followed by strains B10 (5.8 cm) compared to control. However, the decrease of the root length was

recorded by bacterial strains (B17, B18, B20) was (0.5, 0.8, 0.12 cm) respectively whereas, the average root length was recorded by bacterial strains (B8, B9, B11, B12) with (2.5 cm, 2.4 cm, 2.3 cm and 2.3 cm) (Figure 3).

The study of fresh weight indicated an increase of plant growth by the inoculation of lentil seeds with the strain *B11* with about 0.4 g and 0.34 g in the presence of strain *B4.*The increase of the fresh weight compared to the control, was observed in the presence of the isolates *B2* and *B8* respectively. In conclusion, the obtained results indicated that the isolated, selected bacterial strain B13 manifested as excellent bacterial strain for improvement of *Lens culinaris* growth compared to the control and to other treated plants (Figure 3).

Characterization of plant growth promoting traits

Production of indole-3-acetic acid

The production of indole-3-acetic acid by the isolated, selected bacterial strains belonging to the genus of *Bacillus*, inoculated in LB broth supplemented with tryptophan (100µg/ml), incubated at temperature 30°C for 7 days on a rotary shaker (120 rpm), has been achieved by the using of the established calibration curve. The obtained results indicated that the followed isolated, selected bacterial strains *Bacillus* (B3, B15, B20, B16, B18 and B13) have manifested an excellent production of Indole -3-acetic acid in the culture filtrate (Figure 4).

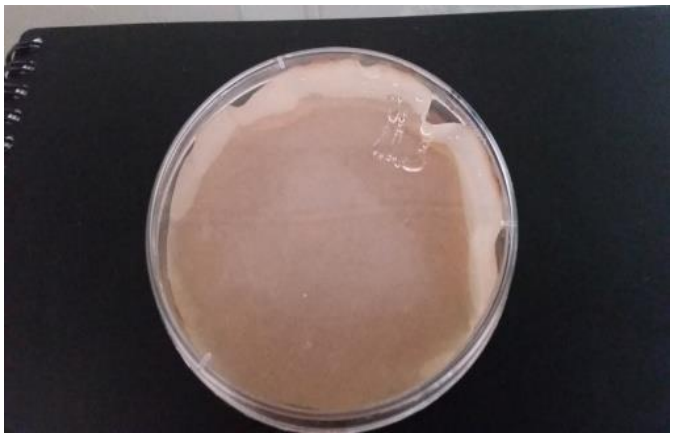

Figure 4: The study of the indol-3-acetic acid production by the isolated, selected phosphate solublization isolate B2 *Bacillus sp*, inoculated on the solid culture medium of Luria Bertani medium in the presence of L-tryptophan, incubated at 28°C for 7 days.

Phosphate solubilization

The exploration of the phosphates solubilization by the isolated, selected bacterial strains belonging to the genus of *Bacillus*, inoculated on the solid culture medium Pikovskaya, which was contained tricalcium phosphate $Ca3(PO_4)_2$, as a sole source of phosphate, incubated at temperature of 30°C for a period of 7 days. The obtained results indicated that both isolated, selected bacterial strains *Bacillus* (B15, B4) has manifested the appearance of the transparency halo around the inoculated colony with solubilizating index between 2.8-3.6 respectively (Figure 5).

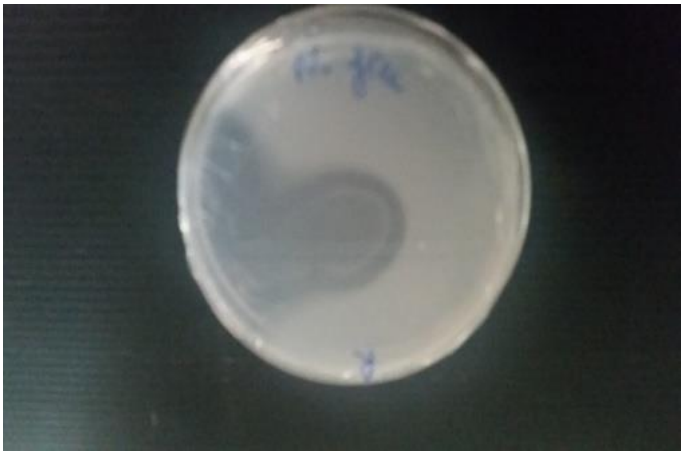

Figure 5: The study of phosphate solubilization by the isolated, selected isolate B15 *Bacillus spp*, inoculated on the solid culture medium of Pikovskaya, incubated at temperature of 28°C for 7 days.

Nitrogen hydrolysis

The nitrogen fixation by the isolated, selected bacterial strains belonging to the genus of *Bacillus* was evaluated by the study of the bacterial growth on the inoculated solid culture free nitrogen culture medium, incubated at temperature of 28°C for 48 hours. The obtained results indicated an important potential of production of ammonia production, manifested by the change of yellow color of the peptone culture medium to brown after the adding of Nessler's reagent.

Figure 6: The nitrogen hydrolyze activity of the isolated, selected *Bacillus sp* B7, inoculated on the solid N-free culture medium, incubated at temperature of 28°C for 48 hours.

Antifungal activity

Twenty four isolated bacterial strains belonging the genus of *Bacillus* were screened for their antagonistic activity against pathogenic fungi *Fusarium oxysporum* by the using well diffusion method. Three isolates (B4, B7, B12) has manifested an excellent antagonistic activity against the investigated pathogenic fungi *Fusarium oxysporum* (Figures 6-8). Furthermore, the

isolates (B1, B2, B3, B10, B15, B16, B17) has indicated an average antagonistic activity against the investigated pathogenic fungi *Fusarium oxysporum.* Moreover, the isolates (B13, B14, B18, B20) has manifested a negligible feeble antagonistic activity against the investigated pathogenic fungi *Fusarium oxysporum.* Finally, the isolates (B5, B8, B11, B11, B14) has manifested a feeble antagonistic activity against the investigated pathogenic fungi *Fusarium oxysporum* with a diameter of inhibition of zone of (25, 20, 20, 14 cm) respectively (Figure 7).

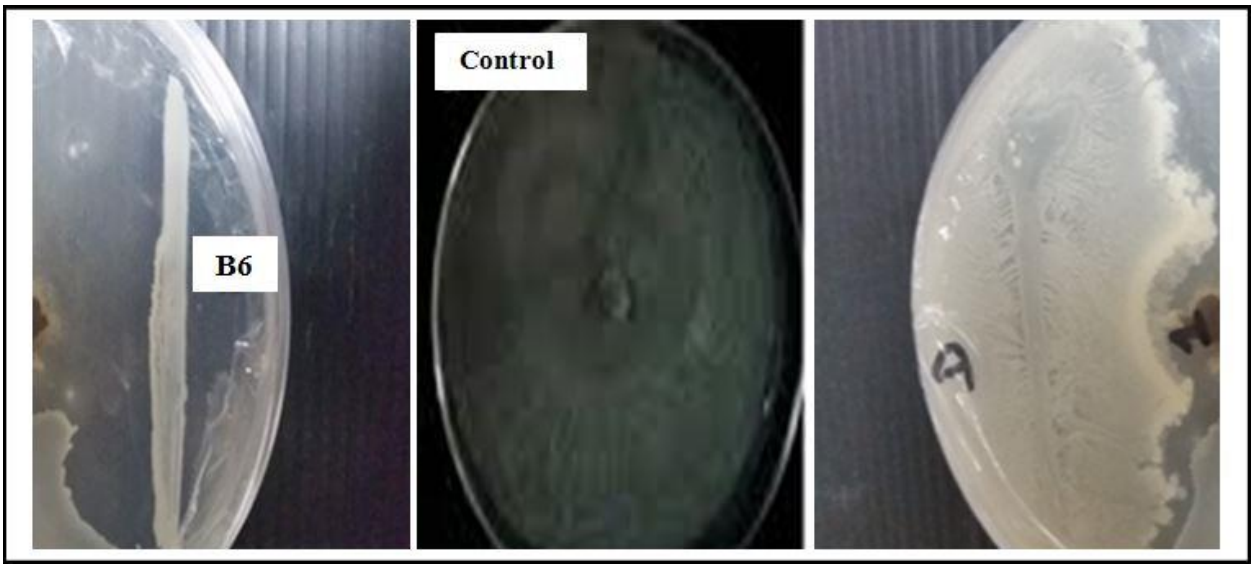

Figure 7: The antifungal activity of the selected phosphate solubilization isolate B6 against *Fusarium oxysporum*, inoculated on the solid culture medium PDA medium, incubated at 28°C for 7 days.

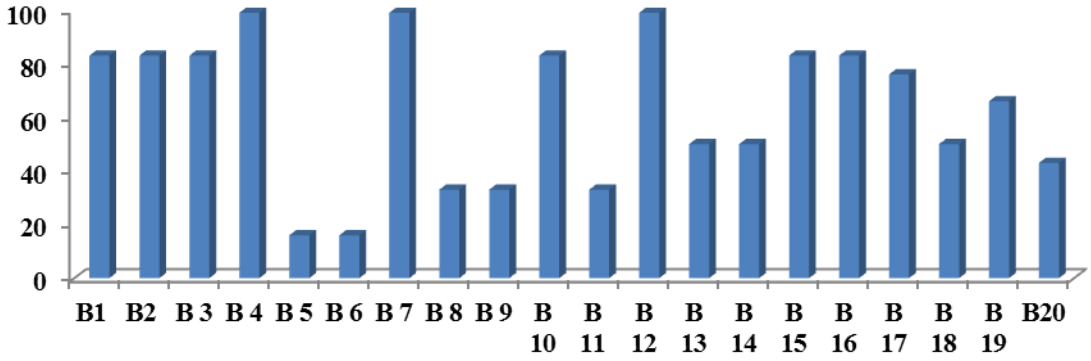

Figure 8: The antifungal activity of the selected phosphate solubilization isolates against *Fusarium oxysporum*, inoculated on the solid culture medium PDA medium, incubated at 28°C for 7 days.

Table 3: Biochemical characters of the isolated, selected bacterial strains promoting growth belonging to the genus of *Bacillus* sp.

Isolates	Nitrogen hydrolysis	Phosphorus solubilization	IAA production	Antifungal activity (% of inhibition)
B3	-	-	+	*5 mm*
B4	+	+/3.6	-	*10 mm*
B5	+	-	-	*25 mm*
B8	-	-	-	*20 mm*
B10	+	-	-	*5 mm*
B11	-	-	-	*20 mm*
B12	-	-	-	*10 mm*
B14	+	-	-	*15 mm*
B15	+	+/2.8	+	*5 mm*
B16	+	-	+	*5 mm*
B17	-	-	-	*7 mm*
B18	-	-	+	*15 mm*

DISCUSSION

The plant growth-promoting rhizobacteria (PGPR) are soil bacteria that colonize plant roots and induced the improvement of bacterial growth by a wide variety of mechanisms. The 4 samples soils, collected from several region of Sidi Bel Abbès, located in North West of Algeria such as Sfisef, Kaid Bel Arbi, ITCMI, and Tessalah, were used for the isolation of the *Lens culinaris* growth-promoting rhizobacteria (PGPR).

Twenty four bacterial strains belonging the of the genus of *Bacillus* were isolated and initially selected by the study of germination process *in vitro* and the determination of some parameters such as root length, stem length, dry weight and fresh grain weight like the (B8, B11, B14 and B15). Furthermore, the isolated, selected promoting growth bacterial strains were subsequently characterized by the study of indol-3-acetic acid (IAA) production, phosphate solubilization, nitrogen assimilation and screened for their antifungal activity against *Fusarium oxysporum*. The availability of the required information such as the diversity and plant growth promotion activity by indigenous *Bacillus* sp isolated from the western region of Algeria. The ability of isolated, selected bacterial strains belonging of the genus of *Bacillus* to colonize the plant roots and to grow under aerobic, anaerobic conditions was a major feature of this bacterial strain.

Furthermore, their capability to overcome the presence of the low oxygen environments constitute a real advantage for bacterial growth in the rhizosphere, where the availability of oxygen can fluctuate over time and diminish [19]. Moreover, the produced indol-3-acetic acid by the isolated, selected bacterial strains belonging of the genus of *Bacillus* was constituted one of many secondary metabolites, which was overproduced abundantly during the stationary bacterial growth phase. The supplemented tryptophan to the culture medium has considerably stimulated the production of the of indol-3-acetic acid by the isolated, selected bacterial strains *Bacillus sp*, which was employed as a main precursor for IAA biosynthesis by bacteria via indole pyruvic acid pathway as described by Patten and Glick [20-22].

Additionally, Patten and Glick [21] has reported that the presence of the low levels of indol-3-acetic acid has stimulated the root elongation, whereas, the high levels of bacterial IAA has stimulated the formation of lateral and adventitious roots. On other hand, the main property of the PGPR that was influenced the plant growth was nitrogen fixation, which was considered as the

principal mechanisms, where the plant benefited from the microbial association. Furthermore, the presence of diazotrophic bacteria constituted a major advantage by nitrogen fixation in exchange for the released carbon as root exudates for plants. In the present work, only 50% of the isolated, selected bacterial strains belonging to the genus of *Bacillus* were characterized by their capability for nitrogen fixation. Guemori-Athmani [23] has reported that the potential of nitrogen fixation by bacterial strains was explored by the investigation of nitrogenase activity by *Bacillus* and *Peanibacillus*, isolated from Algerian soil. Furthermore, Ding [24] has reported that the application of free diazotrophs bacteria, including *Bacillus spp* was considerably increased crops growth.

The presence of several bacterial isolates to solubilize tricalcium phosphate *in vitro* indicated 1the possibility for their application by the crop fields. Rodriguez and Fraga [25,26] has demonstrated that the presence of some genes, which was implicated in the phosphate solubilization by the isolated selected bacteria such as *Bacillus* sp was increased considerably the availability of phosphorus in the soil. The bacterial isolates indicated their potential use for the development of the inoculum in the alkaline soil, based on the solubilization depleted calcium phosphate, which was predominantly present in alkaline soils, where in the acidic soil, phosphate was mainly fixed by Fe or aluminium [27-33]. In the present work, three isolates (B4, B7, B12) has manifested an excellent antagonistic activity against the investigated pathogenic fungi *Fusarium oxysporum* and 6 isolates (B1, B2, B3, B10, B15, B16, B17) has indicated an average antagonistic activity against the investigated pathogenic fungi *Fusarium oxysporum* (Figures 7 and 8).

Rahman [34] has reported that *Bacillus* has a powerful antifungal activity by the producing of a variety of secondary metabolites and hydrolytic enzymes**,** where several bacterial species within genus was able to excrete active lipopeptides. The biological activity of these compounds was mainly related to their effect on the lipids of the cell membrane, where they can promote irreversible pore formation in the double layer of phospholipids, depending on the concentration. These antifungal peptides inhibit the growth of a large number of fungi, such as *Aspergillus, Penicillium, Fusarium*, bacteria and oomycetes [35]. Finally, *Bacillus* species such as *amyloliquefaciens, subtilis, cereus, licheniformis, megaterium, mycoides* and *pumilus* were used in biological control as powerful producers of highly effective antibiotic molecules. Furthermore, the available genomic information of *B. subtilis* indicated that has an average of 4-5% of their genome devoted for of antibiotics biosynthesis, with a production potential of more than 20 structurally diverse antimicrobial compounds [36,37].

CONCLUSION

The main aim of the present study was the isolation and the characterization of the Plant growth-promoting rhizobacteria (PGPR) belonging to the genus of *Bacillus*, which were responsible for the growth of *Lens culinaris*. Twenty four bacterial strains belonging the of the genus of *Bacillus* were isolated and initially selected by the study of germination process *in vitro* and the determination of some parameters such as root length, stem length, dry weight and fresh grain weight like the (B8, B11, B14 and B15). The explored excellent antagonistic activity by the isolated selected bacterial strains (B4, B7, B12) against the investigated pathogenic fungi *Fusarium oxysporum* require a further characterization of the produced enzymes and secondary molecules.

For this purpose, a further studies was required by the purification of the enzyme and a chemical compounds of the secondary metabolite produced by isolated selected bacterial strains (B4, B7, B12), by the using of a modern technique such HPLC, IRM, will require for determination of this active molecules.

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