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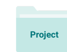

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CHARACTERIZATION OF PLANT GROWTH-PROMOTING MECHANISMS OF RHIZOBACTERIA FROM THE RHIZOSPHERE OF DATE PALMS IN TAFILALET OASES

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This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Tafilalet is the largest oases in Morocco, but it is threatened by climatic and environmental stress like drought, soil degradation as well as plant diseases, in particular Fusarium wilt of date palm caused by *Fusarium oxysporum* f.sp *albedinis* (Foa). Soils are poor in terms of organic matter, nutrient concentrations and biological activity resulting in a dramatic decrease in date palm growth. Improving date palm productivity is regularly achieved by the excessive application of chemical fertilizers, which are harmful for human health and environment. In this work, we aimed at screening the plant growth-promoting rhizobacteria (PGPRs) and at the same time acting as biocontrol agents against Foa. Rhizobacteria associated with date palm and spontaneous plants in Tafilalet oases have been isolated and functionally characterized. Several plant growth promoting and biocontrol traits were investigated, including nutrients solubilization, and the production secondary metabolites and enzymes. Results showed that 97 % of strains was effective in N-fixation, 36 % in ammonia production, 90 % in P-solubilization. The Siderophores and Indol 3-acetic acid production were observed at 30 and 32 strains respectively. Besides, 78 % of strains had an inhibitory effect against Foa, in which 42 % completely inhibited the mycelia growth on PDA medium. Several strains produced cellulase, protease, amylase, chitinase and other secondary metabolites like hydrogen cyanide. Based on these results, consortia of efficient PGPRs could be selected and used as efficient microorganisms for future greenhouse and field experiments.

Keywords: Biocontrol; date palm; fusarium wilt; oases ecosystem; plant growth promoting rhizobacteria.

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1. INTRODUCTION

Following the restoration of the decimated plantations, the Tafilalet oases have recently become an extensive area of date palm groves again, in order to increase the date yields in this area. Before the initiation of the extension program in 2008, farmers did not use chemical fertilizers but only applied agro-ecological practices to ensure their self-alimentation and income under the prevailing harsh environmental conditions like soil and water salinity, irregular and low precipitation, and soil degradation [1,2]. After the launching of the extension program, they started to apply abusively chemical fertilizers, which are not fully assimilated due to the given soil characteristics, in particular low organic matter contents and soil alkalinity [3]. Moreover, Fusarium wilt of date palm also called “Bayoud”, caused by *Fusarium oxysporum* f.sp *albedinis* (Foa), is a serious disease that threatens date palm groves due to the lack of an effective control [4]. However, the application of chemical fertilizers and pesticides did not solve the problems related to the unfavorable soil characteristic and “Bayoud” [4]. Hence, looking for other alternatives became the sole solution to save the oases agroecosystem with their date palms, the main crop in Tafilalet.

One possible alternative, which has recently become increasingly popular, is the use of PGPRs. PGPRs colonize the rhizosphere and have the ability to improve plant growth and/or act as biocontrol agent against pests and diseases [5,6]. Plant growth promotion can be accomplished by several mechanisms. At first, biological nitrogen (N) fixation, a process in which atmospheric N is converted into ammonium by free-living N-fixing rhizobacteria. The ammonium is released into the rhizosphere, where it is taken up by plant roots [7,8]. In addition to N fixation, the solubilization of nutrients is an important mechanism of PGPRs to improve plant nutrition. Phosphorus (P) provisioning can be increased by the release of P from inorganic insoluble phosphates like apatite, or from organic phosphate like inositol phosphate by the secretion of organic acids or phosphatases [9,10].

Also potassium (K) and zinc (Zn) can be solubilized by the release of acids, chelation and exchange reactions [11,12]. In addition, some PGPRs can induce plant growth by stimulating the plants’ own synthesis of auxins [13], gibberellins [14] and cytokines [15] or by the direct production of hormone-like compounds [16,17]. PGPR can also act as biocontrol agents against plant pathogens either by stimulation of plant’s own defense mechanisms, antagonism or antibiosis [18–20]. This is achieved by several mechanisms such as the production of hydrogen cyanide (HCN) [21] or the direct competition for nutrients and space within the rhizosphere [10].

Due to the challenging environmental conditions, arid regions like Tafilalet oases might represent a biodiversity hotspot creating a variety of functionally PGPRs. Thus, we assume that locally isolated PGPRs can promote plant growth and control plant disease even in poor soils. However, efforts to screen a broad range of autochthonous PGPRs from arid region such as oases ecosystems are still missing.

In order to establish a collection of locally-adapted and efficient PGPRs, the study aimed at; (i) isolating rhizobacteria from the rhizosphere of date palms and spontaneous plants in date palm groves of Tafilalet region and perform *in vitro* screenings of their effectiveness as (ii) plant growth promoters and (iii) biocontrol agents against Foa, the causal agent of Fusarium wilt in date palm.

2. MATERIALS AND METHODS

2.1 Sampling Sites

The study area located in Tafilalet region of Morocco is the largest oasis ecosystem of the Sahara desert [22]. The climate is characterized by annual precipitation of 60 to 265 mm from north to south and a mean of 25 rainy days per year. Temperatures range from -1.5 °C (January) to 50 °C (July) with annual mean potential evaporation of 2500 mm [23]. Besides, this region is classified as one of the most arid regions with a low aridity index (0.05 to 0.2), water scarcity and high salinity level [24].

For soil sampling, we selected seven different sites, two traditional and three modern date palm plantations and in addition two undisturbed sites without agricultural activity and covered with the spontaneous plants *Retama blanca* and *Zizyphus lotus* (Table 1). From each site, 1 kg of rhizosphere soil and fresh roots of either date palms or the spontaneous plants was collected from a depth of 10–40 cm using a soil corer. Soil samples were homogenized, sieved at 2 mm, and stored at 4°C until use. A subsample of 500 g was sent to a private laboratory (LaboMag, Casablanca, Morocco) for physical and chemical analysis (soil texture, soil organic matter (SOM) content, pH-value and P content).

Table 1. Coordinates of sampling sites, associated plant species and plantation type

Site N°	Coordinates	Plant species	Plantation type
1	31°42'28.2"N4°08'19.8"W	Retama blanca	Non-agriculturally managed site
2	31°42'28.2"N4°08'19.8"W	Zizyphus lotus	Non-agriculturally managed site
3	31°47'21.9"N4°14'39.0"W	Phoenix dactylifera	Traditional grove
4	31°51'33.2"N4°16'13.5"W	Phoenix. dactylifera	Traditional grove
5	31°41'15.0"N4°54'32.4"W	Phoenix dactylifera	Modern grove
6	31°41'16.0"N4°54'40.5"W	Phoenix dactylifera	Modern grove
7	31°41'12.4"N4°54'36.3"W	Phoenix dactylifera	Modern grove

2.2 Isolation of Rhizobacteria

Ten grams of each soil sample was homogenized in 150 mL Erlenmeyer flasks containing 90 ml of sterile phosphate-buffered saline and shaken at 290 rpm according to the method described by Jäderlund et al.,[25]. Suspensions were serially diluted and 50 µl of each dilution was plated on YPGA (Yeast, Peptone, Glucose and Agar) and King's B media [20]. After incubation, individual colonies were purified and morphologically characterized.

2.3 Screening of Plant Growth-Promoting Traits

2.3.1 Nitrogen fixation and ammonia production

To screen for N fixing potential, the method described by El-Sayed et al. [26] was adopted. Briefly, each bacterial isolate was inoculated by spreading 50 µl on N free medium. At the same time, isolates were inoculated on the same medium supplemented with 0.5 g l^{-1} ammonium sulfate. Isolates continuously growing for 48 at 26 °C after five culturing cycles on N free medium were considered effective for N fixation. Ammonia production was screened according to Ahmad et al., [12]. Bacterial suspensions (3.5 x10^7 colony forming units (cfu) ml^{-1}) were inoculated individually in 50 ml-tubes containing 10 ml of peptone water. After incubation in an incubator-shaker for 96 h at 30 °C, 500 µl of Nessler's reagent was added to each tube. Subsequently, development of yellow to brownish color indicated ammonia production by the isolates. Quantitative analysis was assessed by measuring the optical densities (450 nm) using a spectroscope (UV-mini 1240, Shimadzu). The concentration was estimated based on established curve of commercial ammonium sulfate (Ammonium sulfate *ReagentPlus*®, ≥ 99.0%, Sigma-Aldrich, Saint-Louis, America) [27].

2.3.2 Phosphate, potassium and zinc solubilization

Solubilization of P was assessed according to the method described by Nautiyal [28]. Five µl of bacterial suspension (10^8 cfu ml^{-1}) was inoculated in triplicates on NBRIP agar medium containing inorganic insoluble calcium phosphate ($Ca_3(PO_4)_2$) and incubated for ten days at 28 °C. Solubilization of P results in the appearance of a clear halo around the effective colonies. Phosphorus solubilization index (PSI) was calculated using the formula of Ghosh et al., [29].

$$PSI = (\text{diameter of clear halo and colony (mm)}) / (\text{diameter of clear halo and colony (mm)}) \quad (1)$$

To quantify P solubilization, 100 µl of bacterial suspension (10^8 cfu ml^{-1}) positively tested by the method described above was transferred into 10 ml of liquid NBRIP medium. Cultures were incubated for 10 days at 28 °C and biomass was separated by double-centrifugation (10,000 x g) and microfiltration (0.22 µm). 1 ml of filtrate was added to 4.5 ml of chloromolybdic acid and 25 µl of stannous chloride. Final volume was adjusted to 6 ml with sterile distilled water. The optical density was measured at 600 nm and concentrations were determined using a standard curve of different concentrations of potassium dihydrogen phosphate [30].

To assess K solubilization, 5 µl of bacterial suspension (10^8 cfu ml^{-1}) were inoculated on Aleksandrov solid media containing kaolin as sole K source (pH = 6,5) and incubated for 96 h at 30 °C [31]. Potassium solubilization index (KSI) was also calculated using formula (1). Similarly, Zn solubilization was assessed by inoculating 5 µl of bacterial suspension (10^8 cfu ml^{-1}) on Tris-minimal salts medium supplemented with 14 mM monoxide zinc (ZnO) as sole source of Zn, incubated for 14 days at 28 °C following the method of Fasim et al., [32]. The Zn solubilization index (ZnSI) was also calculated using formula (1). For all solubilization assays, control plates inoculated with sterile distilled water were incubated under the same conditions as the bacterial isolates and each assay was repeated twice.

2.3.3 Siderophore, indole-3-acetic acid and hydrogen cyanide production

Siderophore production was screened by the universal method of Schwyn and Neilands [33] with some

modifications adopted by Taktek [34]. Briefly, 25 ml-tubes containing 10 ml of liquid iron-free medium were inoculated with 100 µl of fresh bacterial suspensions (10^8 cfu ml^{-1}). Cultures were incubated for 96 h in an incubator shaker at 150 rpm and 28 °C, before supernatants were recovered by centrifugation for 10 min at 10000 rpm and 4 °C (MPW 260R centrifuge, MPW med. instruments, Poland). The amount of produced siderophores was quantified by spectrophotometer (UV-mini 1240, Shimadzu) according to Payne [35] slightly modified by Taktek [34]. Briefly, 500 µl of CAS/HDTMA solution (Chrome azurol S-hexadecyl-trimethylammonium bromide) was added to 500 µl of the supernatant and the optical density measured at 630 nm. Siderophores production (US) was calculated as follow:

$$\mathbf{US}(\%) = [(\boldsymbol{Ar} - \boldsymbol{As})/\boldsymbol{Ar}] \times \mathbf{100} \quad \mathbf{(2)}$$

US: Unit of siderophores (in %), Ar: reference absorbance, As: sample absorbance.

Production of indole-3-acetic acid (IAA) was investigated on liquid Czapek medium supplemented with L-tryptophan (200 µg/mL) according to method described by Tsavkelova et al., [36]. Fifteen ml-tubes containing 10 ml of this medium were inoculated with 20 µl of bacterial suspension (10^8 cfu ml^{-1}). After incubation for 72 h at 30 °C in an incubator shaker, supernatant was recovered by centrifugation at 4 °C as described above and 1 ml was supplemented with Salkowski reagent. IAA concentrations were estimated by measuring the optical density at 530 nm and quantified using a standard curve of commercial IAA.

Production of hydrogen cyanide (HCN) was assessed following the method of Wei et al., [37]. In brief, 100 µl of bacterial suspension (10^8 cfu ml^{-1}) was inoculated in Petri dish containing the YPGA solid medium [20] supplemented with glycin. A disc of sterile Whatman paper soaked in picric acid solution was placed on the surface. Cultures were incubated for 96 h at 28 °C and HCN production detected by a color change of the Whatman paper from yellow to reddish brown. For all production assays, uninoculated control plates were incubated under the same conditions as the bacterial isolates and each assay was repeated twice.

2.4 Motility by Swarming

Motility is an important factor for dynamic evaluation of PGPRs and was assessed according to the method of Jahid et al., [38]. In brief, bacterial suspensions (10^8 cfu ml^{-1}) were inoculated (1.5 µl) in the center of nutrient agar plates and incubated for 48 h at 28 °C. The motility was estimated by measuring the diameter of migration.

2.5 Fusarium Wilt Control

The biocontrol effectiveness of our PGPRs was assessed against a pathogenic strain of *Fusarium oxysporum* f.sp albedinis, previously isolated and characterized [39], following the "co-culture method" [10]. One hundred µL of bacterial suspension (10^8 cfu/ml) was individually spread on the Czapek solid medium. In addition, a mycelial agar plug of 5 mm diameter from a 7-day-old culture of Foa was placed on the plate center. Cultures were incubated at 28 °C for 8 days and the inhibition rate (I) on mycelium growth by PGPRs was calculated as follows [40]:

$$\mathbf{I\ (\%) = (\boldsymbol{DC} - \boldsymbol{DT} / \boldsymbol{DC}) \times 100} \quad \mathbf{(3)}$$

DC: fungal growth diameter in the control plate, DT: fungal growth diameter in plate containing the PGPR strain.

To check for mycelia alteration induced by antagonistic PGPRs, a sample of mycelium was observed under a light microscope and characterized as described by Dihazi [40].

To assess the effectiveness of secondary metabolites produced by PGPR strains, the poisoned food technique based on the incorporation of supernatant into culture medium was adopted [41,42]. Briefly, the supernatant of liquid bacterial cultures (prepared in Czapek media) was centrifuged (6000 rpm for 15 min) and filtered (0.22 µm). Subsequently, the supernatant was incorporated into the PDA medium prior to the flow under the laminar flow top. The concentrations were adjusted at 10 % of supernatant for each culture media (PDA). Mycelial agar plugs of 5 mm diameter from a 7-day-old culture of pathogen were placed gently on the plate center and incubated at 28 °C for 6 days. Control plates without supernatant were treated similarly and the assay repeated twice. The inhibition rate was calculated using formula (3).

2.6 Enzymes Activity

Chitinase activity was assessed by inoculating 10 µl of bacterial suspension (10^8 cfu ml^{-1}) on solid media containing colloidal chitin as sole source of carbon [43,44]. Plates were incubated for 96 h at 28 °C and the chitinase activity index was calculated as the ratio of the total diameter (colony + halo zone) and the colony diameter. Amylase activity was assessed by inoculating 10 µl of bacterial suspension (10^8 cfu/ml) on solid media containing starch as sole source of carbon [45]. Plates were incubated for 72 h at 28 °C

and iodine solution was flooded on cultures and the effective isolates were surrounded by a discolored halo. The amylase activity index was calculated as described above. Cellulase activity was assessed based on the cellulose decomposition in the medium containing carboxymethyl cellulose as sole source of carbon and energy [46]. Ten µl of bacterial suspension (10^8 cfu/ml) were inoculated on solid media as described above and incubated at 28 °C for 8 days. After incubation, 0.1 % of Congo red was spread on the plate surface for 15 min followed by washing with a 1 mM NaCl solution. Colonies showing a clear halo indicated the production of cellulase. To assess protease activity, Skimmed milk Agar was inoculated [47], incubated for 72 h at 28°C and protease activity index calculated as described above. For all enzymes activity assays, control plates were inoculated with sterile distilled water and incubated under the same conditions as the bacterial isolates. Each assay was repeated twice.

2.7 Compatibility Screening

Compatibility assays were conducted following the "diffusion through agar method" described by Balouiri et al., [48] to select compatible PGPRs for potential application in PGPR consortia. Based on the screening results, we tested twelve PGPRs of our collection against each other by streaking 100 µl bacterial suspension (10^8 cfu ml^{-1}) of one isolate on YPGA media and placing an agar disk containing another 5 day-old-PGPR strain (from YPGA medium) on the center of the plate. After incubation for 72 h at 28 °C, the presence of an inhibition zone around the agar disc means antagonism between co-inoculated strains. For each assay, control plates was prepared by spreading the bacteria suspension without additional agar disc of another isolate.

2.8 Statistical Analyzes

Data were analyzed using one-way analyses of variance (ANOVA) followed by Duncan's multiple range test with a significance level of α=0.05. Normality of residuals was assessed by Shapiro-Wilk test. All analysis were made using SPSS software (V. 21).

3. RESULTS AND DISCUSSION

3.1 Soil Properties

The study sites were selected based on their contrasting soil properties as presented in Table 2. Most sites were characterized by a pH > 8 and low SOM concentrations (0.7 to 1.3 %) except of sites 5, 6 and 7, with SOM concentrations above 4.8 %. In contrast to the other study sites, these three sites, cultivated under modern management, regularly receive high amounts of manure, i.e. about 50 kg per tree at least twice a year (personal communication). This input of large quantities of organic matter led to an increase in SOM concentrations as compared to the other sites, where farmers rarely use manures for fertilization [49,50].

Most sites contain more than 44 % of sand, especially sites 3 and 4 with 61.2 and 70.2 % respectively. Sites 5, 6 and 7 contained the lowest percentage of sand (31%) but the highest percentage of clay (31 %). The non-agriculturally managed sites hosting the spontaneous plants (site 1 and 2) were characterized by low Olsen P concentrations. Both sites were selected due to their marginal soil properties such as low nutrient and SOM concentrations as described earlier [1,2] with the goal to isolate PGPR strains potentially adapted to extreme conditions. Soil properties of sites 3 and 4 were similar to those previously described in Tafilalet oasis ecosystem where soils are poor in term of SOM and mineral nutrient concentrations [51]. Similarly, Sghir et al., [52] observed a pH of 8.2 and 8.3 and P concentrations of 61 ppm and 8 ppm at site 3 and 4, respectively.

3.2 Rhizobacteria collection

Isolation and purification of PGPRs yielded a total of 33 strains (Table 3). Twenty-six (78 %) strains were isolated from date palm rhizosphere in sites 5, 6 and 7, two strains (6 %), *M09* and *M024* were isolated from sites 3 and 4 respectively, and five (15 %) strains were associated with the spontaneous plants *R. blanca* and *Z. lotus* of site 1 and 2. We found the majority of PGPR strains associated with date palms cultivated under modern agricultural management which involves the application of manure as described above. Several studies have already shown that the application of manure promotes the occurrence of PGPRs in the rhizosphere [53,54]. On the one hand, this is due to the direct input of PGPR coming along with the manure and on the other hand, by providing food for the native PGPRs which promotes their proliferation [55,56]. Ferjani et al., [57] have shown that organic fertilization positively affect PGPR abundance in the rhizosphere of date palms. They investigated PGPR distribution in seven date palm plantations under traditional management in Tunisia, which involves organic fertilization. Using three different cultivation media, TSA (Tryptic Soy Agar), YEM (Yeast Extract Mannitol), and King B, they were able to isolate 440 bacteria. Similarly, Shanthpure et al., [58] observed that organic fertilization increased the population of rhizobacteria

Table 2. Soil characteristics of sampling sites. Data present mean ± standard deviation (n=3)

Site N°	Sand (%)	Silt (%)	Clay (%)	pH	SOM[a] (%)	P[b] (µg/g)
1	45.2 ± 1.8	40.2 ± 1.4	15.6 ± 1.1	8.2 ± 0.2	0.69 ± 0.13	12.4 ± 1.2
2	44.5 ± 1.9	40.2 ± 1.6	15.6 ± 0.9	8.3 ± 0.2	0.70 ± 0.17	12.5 ± 1.2
3	61.2 ± 1.1	29.5 ± 1.0	9.2 ± 0.3	8.2 ± 0.2	1.27 ± 0.03	24.4 ± 1.1
4	70.2 ± 1.3	23.3 ± 1.5	6.4 ± 0.7	8.3 ± 0.3	1.06 ± 0.07	32.4 ± 0.8
5	31.0 ± 0.6	11.7 ± 0.7	31.4 ± 1.0	8.1 ± 0.1	4.90 ± 0.18	37.4 ± 0.6
6	31.0 ± 0.8	11.8 ± 0.6	31.4 ± 1.1	8.2 ± 0.1	4.81 ± 0.24	37.4 ± 0.3
7	31.2 ± 1.7	11.8 ± 0.9	31.4 ± 1.1	8.2 ± 0.2	4.83 ± 0.30	37.3 ± 0.8

[a] Soil Organic Matter, [b] Phosphorus (Olsen)

like *Bacillus*, *Arthrobacter*, *Bradyrhizobium* in a long-term fertilization experiment comparing organic and inorganic fertilizers. In contrast, Choubane et al., [59], who studied PGPR abundance in date palm plantations at a unique site in Oran, Algeria, where SOM levels are low, recovered only 18 isolates using Nutrient Agar media. The abundance of PGPRs depends strongly on the fertilization regime, but also on other factors such as the host plant species, its nutritional status, and plant age [55,60].

3.3 Screening of Plant Growth-Promoting Traits

3.3.1 Nitrogen fixation and ammonia production

We observed that 32 strains (97 %) were effective in N fixation (Table 3) except the strain *M09* isolated from site 3. While 25 N-fixing PGPR strains (75%) were associated with date palm at sites 5, 6 and 7, only one isolate, *M024*, was isolated from site 4. The others were isolated from the rhizosphere of the spontaneous plants (site 1 and 2). In contrast, El-Sayed et al., [26] who isolated rhizobacteria from the rhizosphere of *Capparis spinosa*, *Tribulus terrestris, Citrullus colocynthis, Tamarix amplexicaulis, Glinus lotoides* and *Notoceras bicorne* grown in an arid region of Saudi Arabia, recovered only eight strains (18 %) with the potential for N fixation. Similarly, Mehnaz et al., [61] observed that only nine rhizobacteria (28 %) were able to fix N using the acetylene reduction assay (nitrogenase activity) in the rhizosphere soil of sugarcane in Pakistan. Besides N fixation, we observed twelve of our strains being able to produce ammonia. The concentrations of ammonia produced were statistically different between all our strains ($p < 0.0001$ and $F = 9341.47$) and ranged from 0.2 to 3.0 µmol ml^{-1} with the highest concentration observed for *M015* (Table 3). Ammonia is essential for different biological processes and serves as a precursor for amino acid and nucleotide synthesis [62]. Ferjani et al., [57] have shown that ammonia production is the second most abundant trait found in rhizobacteria associated with the root system of date palm cultivated in seven oases in Tunisia. Also, Laslo et al., [10] reported that 37 PGPR isolates from a total of 47 isolates, were able to produce ammonia.

3.3.2 Screening of phosphorus, potassium and zinc solubilization

Phosphorus availability in the soil is a key factor for plant nutrition and growth. However, calcium phosphate, which is formed when calcium is highly dominant in soils, as particularly found in arid ecosystems [51], is not plant available. Thus, its solubilization by PGPRs is a valuable trait in the alkaline soils of date palm plantations. Among our collection, 90 % of our strains were able to solubilize tricalcium phosphate with PSI ranging from 0.6 (*M016*) up to 2.1 (*M010*) ($p < 0.0001$ and $F = 3697.11$) (Table 3). Quantitative analyzes in liquid media revealed that P solubilization significantly differed among strains ($p < 0.0001$ and $F = 4272.34$) and ranged from 2.8 to 591.8 µg P ml^{-1} released by *M016* and *M010,* respectively. Few strains like *M012*, *M013* and *M014* could only solubilize P in liquid medium but not on solid medium. This might be due to low diffusion rates of organic acids secreted by those strains on solid media as already described by Johnston and Freiser [63]. As reported by Oliveira et al., [64] as well as Bhattacharyya and Jha [9], mechanisms by which PGPRs solubilize phosphate include the production of organic acids, phytases and phosphatases. Fankem et al., [65] isolated ten PGPR strains from the rhizosphere and root fragments of oil palms in Cameroun and observed highest PSI of 2.33, 2.48 and 2.40 when using Nutrient Agar amended with calcium phosphate ($Ca_3(PO_4)_2$), aluminium phosphate ($AlPO_4$) and iron phosphate ($FePO_4$) as sources of inorganic phosphate, respectively [65]. Besides, it was shown that phosphate solubilizing-bacteria are more abundant in the rhizosphere zone of *Salix alba* as compared to bulk soil zone [66]. In addition, this study showed that the PSI and soluble P-concentrations strongly depended on the media used for PGPR screening and that the best medium is the NBRP and MPVK (modified Pikovskaya) media.

Besides P, also K availability is often very limited under marginal soil conditions. Among our collection, 17 strains (51 %) were effective in K solubilization. Indexes significantly differed among strains ($p < 0.0001$, F= 3766.027) and ranged from 1.0 (*M09*) to 2.7 (*M026*) (Table 3). It has been shown that K solubilization by PGPR in Aleksandrov medium was dependent on the pH-value of the medium and the K source i.e. aluminum potassium silicate ($AlKO_6Si_2$) or kaolin [67]. Thus, the lack of K solubilization by some strains might be due to potentially suboptimal conditions for the respective strains or by limited release rates of organic acids into the solid medium as reported previously [28]. In addition, using the same medium (Aleksandrov medium) and waste mica as sole K mineral, researchers have isolated twelve effective PGPR (among 30 isolates) from maize, banana, sugarcane, potato, pigeon pea, and tobacco [68]. Setiawati and Mutmainnah [31], who isolated PGPR strains from sugarcane rhizosphere observed that 15 out of 45 strains were able to solubilize K using different K sources (Feldspar, trachyte, Leucite Pati, Leucite Situbondo). The authors reported about highest KSI of more than four on Aleksandrov medium supplemented with Feldspar, leucite or trachyte, and claimed that the KSI greatly depend on the mineral source and PGPR strains.

A high quantity of inorganic Zn in soils is converted into unavailable forms such as zinc oxide (ZnO) or zinc phosphate [Zn3(PO4)2·4H2O] [11,69]. Hence, inoculation of Zn solubilizing PGPRs into the rhizosphere to increase Zn assimilation and growth of crop plants has proven to be promising [69]. In our study, we recovered six strains (18%) effective in ZnO solubilization, all isolated from date palm rhizosphere of modern plantations (sites 5, 6 and 7). Solubilization indexes differed between strains ($p < 0.0001$, F = 17713.67) and ranged between 2.3 (*M017*) and 3.1 (*M08*). Yaish et al.,[70] who studied endophyte communities of date palms in Oman, observed that strains isolated from four soil samples were able to solubilize K from mica and Zn from ZnO and could enhance root elongation of canola under salt stress as well. Manasa S. G. et al., (2019) isolated 40 zinc solubilizing PGPR using a TMA medium (TRIS-minimal agar medium) supplemented with ZnO (0.1%) with a maximum ZnSI of 21 mm. In India, eight Zn- and four K-solubilizing rhizobacteria (from a total of 14 isolates) were isolated from maize, rice and cotton rhizosphere using ZnO and aluminum potassium silicate as mineral sources [72]. A study conducted in Saudi Arabia reported about 37.63% of rhizobacteria associated with the rhizosphere and rhizoplane of elven spontaneous plants to be able to solubilize Zn from ZnO [26].

3.3.3 Siderophores, IAA and HCN production and swarming motility

Among our collection, 30 strains were able to produce siderophores (Table 3), and significantly differed regarding their production rates ($p < 0.0001$, F = 8.212) and ranged between 11.9 % (*M018*) and 61.9 % (*M015*). Similarly, in the southern region of Tunisia, where aridity is one of the major factors strongly influencing the functionality and diversity of rhizobacteria associated with date palm, researchers showed that siderophores production is the fourth common trait in PGPR [57]. Siderophore production rates ranged from 20 to 62 % depending on site characteristics from where the PGPRs were isolated from. Similarly, El-Sayed et al., [26] have shown that spontaneous plants in arid regions of Saudi Arabia are niches for siderophores producing rhizobacteria. Their strains possessed siderophores production rates ranging between 10 % and 64 % depending on the sampling site.

The role of siderophores in plant growth promotion and control of fungal diseases was previously described [73–76]. When iron is limited, the secretion of siderophores by PGPRs can promote plant growth under stressful conditions [77]. Since the majority of PGPRs produce siderophores while possessing a high affinity to plants, they can protect these plants against a wide range of pathogens [75]. It has been suggested that differences between PGPRs in term of siderophores production, might depend on environmental conditions of the sampling site such as climate, soil properties and crop species as well as on the genus and PGPRs species [78].

In this work, 32 PGPR strains were able to produce IAA (Table 3). Production of IAA significantly differed between strains ($p < 0.0001$ and F = 4375.29) and ranged between 3.8 and 61.8 µg IAA ml^{-1} with *M020* being the most productive PGPR strain. Indole-3-acetic acid is a kind of auxins that is responsible for division, extension, and differentiation of plant cells and tissues and stimulate seed germination, control processes of vegetative growth, tropism, florescence, and fructification of plants [79]. Studies investigating IAA production in rhizobacteria associated with plants growing in arid regions of India showed that IAA amounts produced did not exceed 42 µg IAA ml^{-1} [74,80]. In another study, the *Pseudomonas fluorescens* 002 (P.f.002.), associated with date palms growing in the Algerian Sahara (Ghardaia region) was characterized as IAA producing PGPR. In contrast to our PGPR strains, P.f.002 was only able to produce IAA concentrations of 1.05 µg IAA ml^{-1} when it was incubated in the Czapek medium amended with 1 mM Tryptophan for 24 h at 30 °C [81]. One reason for the

high IAA concentration observed for some of our PGPR strains might have resulted from the longer incubation period of 72 h compared to 24 h, even though the tryptophan concentration added to the our Czapek media was similar (0.98 mM). According to Tsavkelova et al., [36], 0.98 mM is the optimal concentration for IAA production such as applied in our study. Thus, the best PGPR strains was *M021* as indicated above.

The production of HCN was low to moderate for all productive strains (15 %). *M010* and *M013* isolates showed the highest HCN production as reflected by the higher color intensity of the Whatman paper (Table 4). Similarly, El-Sayed et al., [26] observed that only 6.5 % of their PGPRs strains were able to produce HCN in TSA media supplemented with glycine and picric acid and that their efficiency was related to the plant species and microhabitat from which their PGPRs were isolated from. This low number of effective isolates could be due to extreme climatic conditions such as reported by [82] who could not detect any HCN producing strain among cacti-associated rhizobacteria isolated from Brazilian semi-arid regions. It has been suggested that the limited availability of the amino acid glycine in the rhizosphere of study plants could be the reason for the low abundance of HCN producing rhizobacteria [61]. This might also be the reason why we only observed few PGPR strains able to produce HCN. The production of volatile compounds, including HCN is an important trait of PGPRs and contribute to the control of phytopathogens by blocking the transfer of electrons and altering the functioning of cytochrome oxidase in the respiratory chain and the energetic potential of pathogens [83]. Thus, such PGPR strains can effectively compete with root pathogens and consequently improve plant growth [84].

Swarming motility is another important factor to select for PGPRs with a higher motility for a more effective colonization of the rhizosphere [85]. Screening of swarming motility (Table 4) revealed that 27 isolates (81%) showed different efficiencies of movement on semi-solid medium ($p < 0.0001$, $F = 3293.63$). Strains *M013*, *M08*, *M01* and *M021* were the most mobile ones reaching 86.2, 86.3, 85.5 and 85.5 mm diameter respectively, while *M05* was the slowest one reaching only 7.5 mm. The motility depends generally on the type of mobility agents, its disposition as well as involved genes or proteins as revealed in mutant strains of *Pseudomonas aeruginosa* which lose their motility [86,87]. Similarly, Chebotar [88] described that the motility depends on the PGPR species and the mobility agent used for the assay.

3.4 Fusarium Wilt Control

Among our cultures, 26 strains (78.8 %) exerted antifungal activity against Foa on solid media (Table 4) with significant differences between the strains ($p < 0.0001$, $F = 844.81$). Fourteen isolates (42.4 %) showed an inhibition rate (IR) of 100 % including *M017, M018, M021* and *M030*. All strains associated with spontaneous plants (sites 1 and 2) had an inhibitory effect on Foa with two strains which completely inhibited its growth. Inhibition rates (IR) observed for other PGPR stains ranged between 6.5 % (*M016*) and 94.1 % (*M028*). In parallel, we also observed cytological alterations (e.g. vacuolization, enlargement and swelling) of Foa mycelia when co-inoculated with some antagonistic PGPR strains (results not shown). Using the supernatant of our cultures we observed 28 of our PGPRs (84.9 %) to inhibit the growth of Foa mycelia (Table 4) with a significant difference in efficiency between the strains ($p < 0.0001$, $F = 227.06$). The supernatant of *M014* and *M030,* originally associated with *Zizyphus lotus* showed the highest inhibition rates (SI) of more than 65 %. However, *M025*, *M08* and *M028* exerted growth inhibition rate less than 16 %. Similarly, El Arbi et al., [89] isolated PGPRs associated with date palm grown in the oasis ecosystem in Tunisia and screened their biocontrol potential against Foa. In their study, only two *Bacillus* spp. effectively inhibited Foa growth most likely due to the production of surfactin, fengycin or iturin. In addition, siderophores, HCN and enzyme production are other mechanisms by which PGPRs can antagonize fungal pathogens [90,91]. Adesina et al., [92] showed that 11.5 % of rhizobacteria isolated from soils known for their suppressive effect against phytopathogens, effectively inhibited under *in vitro* conditions *F. oxysporum*. These antagonist rhizobacteria were isolated from six locations in Europe and have been shown to produce siderophores and several lytic enzymes including chitinase, cellulase and protease [92]. Another study has shown that six from a total of 14 rhizobacteria, associated with maize plants, were able to inhibit *Fusarium verticillioides*, a pathogenic fungus of *Z. mays* in Benin. The highest inhibition rate was only 52.24% on PDA medium (Potato Dextrose Agar) using the dual culture method [93]. In contrast, all rhizobacteria, isolated from the rhizosphere of common bean and screened for their ability to inhibit plant pathogenic fungi, effectively inhibited *F. oxysporum* and *F. solani* with maximum inhibition rates of 65.91 % and 64.38 %, respectively [94].

Table 3. Results of plant growth-promoting traits. Data present mean ± standard deviation, n = 6; +, fixation; Nd = non-detected

Strain	Site	N-fix [c]	NH_3 [d] (µmol ml^{-1})	PSI [e]	P (µg ml^{-1})	KSI [f]	Sid (%) [g]	IAA (µg ml^{-1}) [h]	ZnSI [i]
M01	5	+	2.5 ± 0.1b	1.5 ± 0.1	185.5 ± 2.7	Nd	22.6 ± 2.1	31.4 ± 0.3	3.1 ± 0.1
M02	7	+	Nd	1.2 ± 0.1	359.6 ± 1.7	Nd	42.1 ± 2.4	42.1 ± 1.1	Nd
M03	5	+	Nd	1.9 ± 0.1	357.6 ± 2.8	Nd	15.0 ± 1.5	4.7 ± 0.1	Nd
M04	2	+	2.2 ± 0.1	1.6 ± 0.1	258.8 ± 4.5	1.6 ± 0.2	33.1 ± 1.6	39.7 ± 0.3	Nd
M05	1	+	0.2 ± 0.1	1.8 ± 0.1	385.9 ± 2.9	1.6 ± 0.3	29.9 ± 2.1	13.4 ± 0.1	Nd
M06	5	+	Nd	1.9 ± 0.2	258.4 ± 3.9	1.4 ± 0.1	40.0 ± 1.0	16.8 ± 0.2	Nd
M07	5	+	Nd	1.7 ± 0.1	320.2 ± 1.5	Nd	36.2 ± 1.8	9.7 ± 0.2	Nd
M08	5	+	2.2 ± 0.1	1.8 ± 0.1	313.5 ± 2.2	Nd	33.0 ± 1.0	16.2 ± 0.1	3.1 ± 0.1
M09	3	0	Nd	1.7 ± 0.2	455.8 ± 1.9	1.0 ± 0.1	28.1 ± 2.6	14.4 ± 0.3	Nd
M010	5	+	2.5 ± 0.1	2.1 ± 0.1	591.8 ± 1.5	2.2 ± 0.2	50.9 ± 4.8	40.8 ± 0.2	Nd
M011	7	+	Nd	1.3 ± 0.1	158.4 ± 1.7	Nd	40.2 ± 0.2	7.6 ± 0.1	Nd
M012	7	+	2.6 ± 0.1	Nd	58.5 ± 2.5	Nd	12.6 ± 2.1	22.4 ± 0.3	Nd
M013	6	+	1.9 ± 0.1	Nd	98.7 ± 2.7	1.9 ± 0.1	24.3 ± 0.5	4.2 ± 0.1	Nd
M014	2	+	1.8 ± 0.1	Nd	70.3 ± 1.6	1.5 ± 0.2	22.5 ± 1.8	8.3 ± 0.1	Nd
M015	6	+	3.0 ± 0.1	1.6 ± 0.1	319.0 ± 1.9	1.5 ± 0.4	61.9 ± 4.0	4.9 ± 0.1	Nd
M016	7	+	Nd	0.6 ± 0.1	2.8 ± 0.1	Nd	44.7 ± 2.1	32.4 ± 0.1	Nd
M017	7	+	Nd	1.6 ± 0.1	170.3 ± 2.2	1.4 ± 0.2	21.9 ± 2.7	3.8 ± 0.1	2.3 ± 0.1
M018	2	+	Nd	1.4 ± 0.1	392.2 ± 1.7	1.1 ± 0.1	11.9 ± 2.1	21.0 ± 0.5	Nd
M019	6	+	1.9±0.1	1.2 ± 0.2	384.2 ± 2.2	1.8 ± 0.3	35.9 ± 19.4	27.3 ± 0.1	Nd
M020	6	+	2.0±0.1	1.9 ± 0.1	311.8 ± 1.4	Nd	53.7 ± 15.3	61.8 ± 0.1	Nd
M021	5	+	Nd	1.7 ± 0.1	4.9 ± 0.6	2.7 ± 0.2	14.7 ± 0.9	46.5 ± 0.3	Nd
M022	7	+	Nd	1.2 ± 0.1	191.9 ± 3.0	Nd	44.6 ± 2.3	6.3 ± 0.2	Nd
M023	7	+	Nd	1.4 ± 0.1	192.3 ± 2.1	1.2 ± 0.1	56.6 ± 1.3	41.3 ± 0.1	2.7±0.1
M024	4	+	Nd	1.9 ± 0.1	467.7 ± 1.6	Nd	46.2 ± 0.6	4.3 ± 0.1	Nd
M025	6	+	2.1±0.1	1.8 ± 0.1	242.7 ± 2.4	Nd	53.2 ± 3.1	30.3 ± 0.5	Nd
M026	7	+	Nd	1.4 ± 0.1	261.8 ± 1.6	2.7 ± 0.1	35.3 ± 1.4	46.8 ± 0.1	Nd
M027	7	+	Nd	1.6 ± 0.2	245.7 ± 2.2	Nd	35.8 ± 1.4	4.4 ± 0.2	Nd
M028	7	+	Nd	1.3 ± 0.2	22.9 ± 0.9	2.6 ± 0.2	48.5 ± 0.6	12.1 ± 0.1	Nd
M029	5	+	Nd	1.6 ± 0.1	312.6 ± 1.3	2.6 ± 0.3	25.5 ± 1.5	4.5 ± 0.1	2.9±0.1
M030	2	+	Nd	1.5 ± 0.3	Nd	Nd	59.8 ± 4.1	4.5 ± 0.1	Nd

Strain	Site	N-fix [c]	NH_3 [d] (µmol ml^{-1})	PSI [e]	P (µg ml^{-1})	KSI [f]	Sid (%) [g]	IAA (µg ml^{-1}) [h]	ZnSI [i]
M031	5	+	Nd	Nd	Nd	1.9± 0.1	Nd	10.8 ± 0.1	2.5±0.1
M032	7	+	Nd	Nd	Nd	Nd	Nd	Nd	Nd
M033	5	+	Nd	Nd	Nd	Nd	Nd	51.7 ± 0.1	Nd

[c] Nitrogen fixation, [d] Ammonia production, [e] P- solubilization index, [f] K- solubilization index, [i] Zn-solubilization index, [g] Siderophores production, [h] Indole 3-acetic acid

Table 4. Results of plant growth-promoting traits and antifungal activities. Data present mean ± standard deviation, n = 4. ++, high production; +, moderate production; Nd= non-detected

Strain	Site	HCN [j]	Motility (mm) [k]	IR (%) [l]	SI (%) [m]	Amylase [n]	Cellulase [o]	Chitinase [p]	Protease [q]
M01	5	Nd	85.5 ± 0.5	39.3 ± 2.2	41.3 ± 1.0	Nd	Nd	Nd	+
M02	7	Nd	65.3 ± 0.7	100 ± 0.0	54.2 ± 3.9	Nd	Nd	Nd	Nd
M03	5	Nd	55.2 ± 0.2	77.5 ± 1.2	61.7 ± 0.8	Nd	Nd	Nd	Nd
M04	2	Nd	20.8 ± 0.1	87 ± 0.5	48.5 ± 1.5	1.7 ± 0.1	3.3 ± 0.0	Nd	+
M05	1	Nd	7.5 ± 0.5	26.6 ± 1.9	55.6 ± 5.2	Nd	Nd	Nd	Nd
M06	5	+	76.5 ± 0.5	36.67 ± 2.6	31.9 ± 1.7	Nd	Nd	Nd	Nd
M07	5	Nd	Nd	Nd	26.4 ± 2.1	Nd	Nd	Nd	Nd
M08	5	Nd	86.3 ± 0.7	100 ± 0.0	9.0 ± 0.7	Nd	Nd	1.4±0.2	Nd
M09	3	Nd	60.7 ± 0.2	Nd	57.9 ± 1.0	1.7 ± 0.1	Nd	Nd	++
M010	5	++	52.6 ± 0.5	100 ± 0.0	51.2 ± 1.6	Nd	Nd	Nd	+
M011	7	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
M012	7	Nd	68.9 ± 0.2	100 ± 0.0	50.0 ± 1.4	Nd	1.6 ± 0.1	Nd	Nd
M013	6	++	86.2 ± 0.2	63.5 ± 0.3	26.4 ± 2.3	1.3 ± 0.01	1.9 ± 0.2	Nd	Nd
M014	2	Nd	67.5 ± 0.5	48.8 ± 0.3	66.0 ± 0.7	1.3 ± 0.1	3.2 ± 0.1	Nd	Nd
M015	6	Nd	54.9 ± 0.1	100 ± 0.0	44.4 ± 1.4	nd	Nd	Nd	+
M016	7	Nd	67.1 ± 0.5	6.5 ± 0.5	25.3 ± 1.7	1.3 ± 0.1	1.1 ± 0.2	Nd	Nd
M017	7	Nd	37.3 ± 0.5	100 ± 0.0	20.3 ± 0.5	Nd	1.8±0.1	Nd	Nd
M018	2	Nd	37.2 ± 0.2	100 ± 0.0	29.2 ± 1.4	Nd	nd	Nd	Nd
M019	6	+	72.2 ± 0.2	87.6 ± 0.7	15.3 ± 0.4	Nd	2.3± 0.01	Nd	Nd
M020	6	Nd	20.5 ± 0.5	54.5 ± 2.3	51.2 ± 0.6	Nd	Nd	Nd	Nd
M021	5	Nd	85.5 ± 0.5	100 ± 0.0	32.8 ± 0.8	Nd	Nd	1.4 ± 0.1	+
M022	7	Nd	77.0 ± 0.7	100 ± 0.0	57.1 ± 0.2	2.7 ± 0.3	3.8 ± 0.2	Nd	Nd
M023	7	Nd	64.0 ± 0.1	100 ± 0.0	52.1 ± 0.7	Nd	Nd	Nd	Nd
M024	4	Nd	44.7 ± 0.7	100 ± 0.0	28.1 ± 0.3	Nd	Nd	Nd	++
M025	6	+	34.2 ± 0.2	100 ± 0.0	09.4 ± 1.0	Nd	Nd	Nd	+

Strain	Site	HCN [j]	Motility (mm) [k]	IR (%) [l]	SI (%) [m]	Amylase [n]	Cellulase [o]	Chitinase [p]	Protease [q]
M026	7	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
M027	7	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
M028	7	Nd	32.3 ± 0.5	94.1 ± 0.5	16.0 ± 0.7	Nd	Nd	Nd	Nd
M029	5	Nd	Nd	Nd	Nd	Nd	Nd	Nd	+
M030	2	Nd	31.5 ± 0.5	100 ± 0.0	65.5 ± 1.1	1.7 ± 0.1	3.4 ± 0.1	Nd	Nd
M031	5	Nd	81.5 ± 0.5	47.6 ± 2.0	29.9 ± 0.7	Nd	nd	2.7 ± 0.2	Nd
M032	7	Nd	Nd	Nd	Nd	Nd	nd	Nd	Nd
M033	5	Nd	24.5 ± 0.5	100 ± 0.0	51.0 ± 1.7	1.4 ± 0.1	2.3 ± 0.1	Nd	+

[j] Hydrogen cyanide production, [k] Swarming motility [l] Inhibition rate by antagonism, [m] Inhibition rate by supernatant (secondary metabolites) [n] Amylase index, [o] Cellulase index, [p] Chitinase index and [q] Protease production

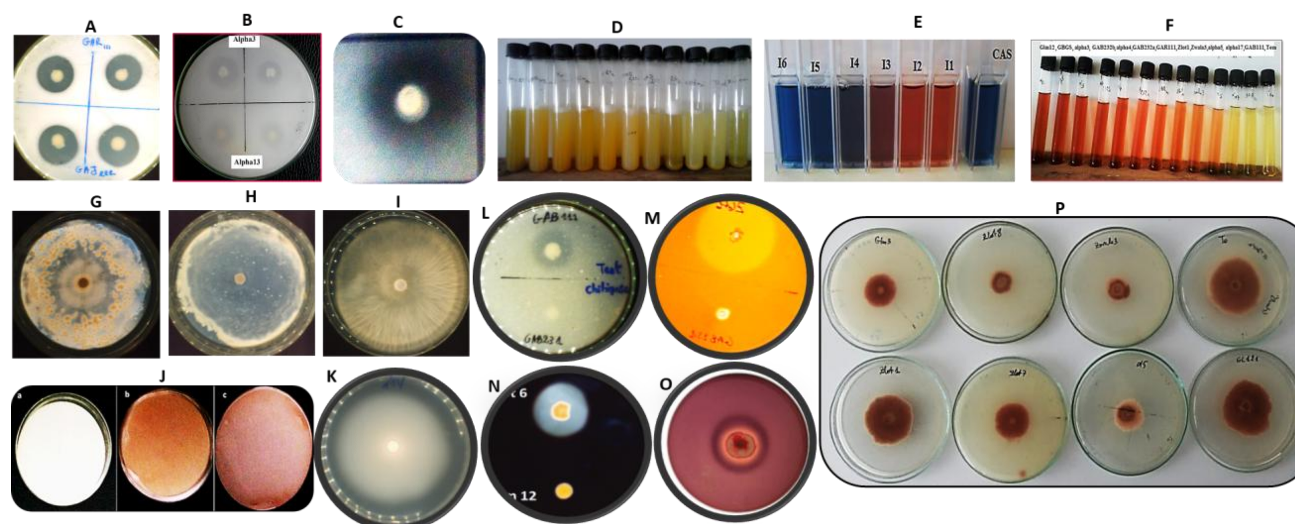

Fig. 1. Plant growth traits of rhizobacteria. A) Zn solubilization on solid medium, B) K solubilization on solid medium (with Kaolin solution), C) phosphorus solubilization on solid NBRIP medium, D) Ammonia production on liquid media, E) Siderophores production in CAS-HDTMA liquid media, F) IAA production in liquid Czapek media, G, competition towards Foa with partial inhibition, H) competition towards Foa with a complete inhibition (rate of 100 %), I) competition towards Foa without inhibition (rate of 0%), j) HCN production on YPGA medium added with Glycin, K) motility by swarming, L) chitinase production, M) cellulase, N) Amylase, O) Protease, P) effect of supernatant on Foa growth in PDA media

3.5 Enzyme Activities and Compatibility

Amylase production was detected in eight strains (24 %) which differed significantly in their efficiency ($p < 0.0001$, $F = 175.04$). *M022* exhibited the highest activity with an index of 2.7, while *M014* and *M013* had the lowest activity with an index of 1.3 (Table 4). Besides, three isolates (9 %) were able to produce chitinase with a significant difference in term of their efficiency ($p < 0.0001$, $F = 1795.11$). The highest index of 2.7 was observed for *M031* and the lowest index of 1.4 for *M08*. Cellulase activity was detected in five strains (14 %) which differed significantly in their efficiency ($p < 0.0001$, $F = 2714.50$) with indexes ranging from 1.1 to 3.8 and *M022* being the most efficient strain. Protease activity was detected in eleven strains (32 %) with *M024* and *M09* being more efficient as compared to the other strains (Table 4). All these enzymatic activities are important for the characterization of PGPRs and represent fundamental functions of biocontrol agents and plant growth promoters [95,96]. Amylase has been considered a plant growth-promoting trait, as it hydrolyzes starch and thus provides energy for the growth of root and shoots [83]. In Algeria, rhizosphere of some plants species, including date palm and carob tree were sampled and 18 amylase producers were found to be associated with date palm with an index ranged between 1.65 and 2.16 [59]. Similarly, [96] studied endophytic bacteria in Jouli oasis in Tunisia, associated with date palm roots growing in soils with similar characteristics to ours. In contrast to our results, most of their PGPR strains proved to be effective in terms of amylase (38.46%), protease (69.23%) and cellulase (76.92%) activity.

A study conducted in seven oases in Tunisia, observed that the efficiency of cellulase and protease activity of PGPR strains isolated from date palms were strongly depended to the sampling site and the PGPR species [57]. Another study conducted in the arid region of Saudi Arabia, showed that protease activity was the most commonly shared enzymatic trait between all isolated PGPR strains (23.7 %), followed by chitinase activity (13.9 %) and cellulase activity (10.7 %) [26]. These functional traits might represent crucial factors to control against fungal pathogens in the rhizosphere and thus to promote plant growth. Indeed, when enzymatic activities (cellulase and laminarinase) and the population of pathogen *Phytophtora cinnamomi* were measured in upper and lower soil depths of 15 cm, enzyme activities were positively correlated with the population of saprophytic fungi and negatively with the population of *Phytophtora cinnamomi* (the causal agent of root rot disease) and rot incidence in root of avocado [97]. Similarly, it was found that *Bacillus amyloliquefaciens* that synthesized proteases exerted a direct inhibitory effect on fungal growth, including *Fusarium oxysporum* species, by degrading fungal cell walls [83,98].

Finally, compatibility tests are a crucial trait to select efficient PGPR strains, which will be combined in consortia to be used in greenhouse and/or field experiments. Among twelve PGPRs strains, 10 combinations have shown antagonism on solid media as revealed by an inhibition zone between incompatible strains (Table 5). These inhibitory effects of bacteria toward each other could be due to the secretion and diffusion of antibacterial substances such as antibiotics [99], bacteriocins, organic acids [100], cyanide hydrogen, siderophores or enzymes [93] .

Table 5. Compatibility results of twelve rhizobacteria. NC: incompatible combination and C; compatible combination

Strain	M01	M04	M05	M010	M012	M013	M014	M015	M019	M020	M025	M08
M01												
M04	C											
M05	C	C										
M010	C	NC	C									
M012	C	NC	C	C								
M013	NC	C	C	C	C							
M014	C	C	C	C	C	C						
M015	C	C	C	C	NC	C	C					
M019	C	C	C	NC	C	C	C	C				
M020	C	C	C	NC	NC	NC	C	C	NC			
M025	C	C	C	C	C	C	C	C	C	C		
M08	C	C	C	C	C	C	C	C	C	C	NC	

4. CONCLUSIONS AND PERSPECTIVE

A collection of PGPR strains was recovered from the rhizosphere of date palms and spontaneous plants collected from Tafilalet oases under stressful conditions including soil salinity, alkalinity and drought. A series of plant growth promotion and biocontrol traits against Fusarium wilt disease were investigated to functionally characterize PGPR strains potentially adapted to the harsh conditions of arid oases ecosystems. The screening of Plant growth promoting traits have revealed the richness of efficient PGPR in term of nutrient solubilization as well as IAA, siderophores, ammonia and enzymes production. In addition, we observed that the majority of our PGPR strains efficiently antagonized the *Fusarium oxysporum* f.sp *albedinis* via competitions and antibiosis and that 42% even completely inhibited its growth. Based on these results, consortia of efficient PGPRs with contrasting plant growth promotion and biocontrol traits were selected for *in vivo* inoculations to further test their efficiency in pot and field trials. Currently, we carry out these experiments which might be a biological alternative to improve the supply of nutrients for date palms and control Fusarium wilt, a serious disease that heavily threatens date palm worldwide.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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