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Research Article

Characterization and Identification of Plant Growth Promoting Traits of a Rhizobacteria: Pantoea Agglomerans 20-19

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Abstract

Plant Growth Promoting Rhizobacteria (PGPR) are known to influence positively plant growth by various direct or indirect mechanisms. The objective of this study was to characterize and identified a bacterium was isolated from the wheat rhizosphere of an semi arid area Meknes (Morocco). The studied strain possess several PGPR trais such as nitrogen fixation ability, phosphate solubilization, production of auxins and Ammonia production. Based on their 16S rDNA sequences, the strain were identified as *Pantoea agglomerans* 20-19. This *Pantoea agglomerans* 20-19 is capable of IAA, ammonia production, nitrogen fixation and solubilization phosphorus. These results showed that *Pantoea agglomerans* 20-19 with its PGPR traits could consitute a good biofertilizer in semi arid area.

Keywords: *Pantoea agglomerans*; PGPR; IAA; Ammonia; Nitrogen fixation and phosphorus solubilization

Introduction

Indiscriminate use of chemical fertilizer and pesticides over the last few decades has not only resulted in the contamination of environment, but also reduced soil fertility. However, the fertilizers overuse could lead to serious soil acidification, nutritional imbalance and deterioration of the rhizosphere micro-ecological environment, further increased the activity of heavy metal ions in soil [1]. Due to the adverse effects of chemical fertilzers on the environment and ecology, nowadays bio-fertilizers (Plant growth promoting rhizobacteria) are being projected as an essential component of organic farming to play a key role in the maintenance of long-term soil fertility as well as sustainability [2].

The rhizosphere microbiome harbors Plant Growth Promoting Rhizobacteria (PGPR), nitrogen fixing symbionts, endophytes, mycorrhizal fungi, biocontrol microorganisms [3-5]. PGPR are a group of bacteria that colonize plant roots and proivde beneficial effects on plant growth and development. PGPR have direct or indirect effects on plant growth promotion and improved crop yield. Direct effects of PGPR include providing plants with fixed nitrogen and phytohormones, increasing the availability of nitrogen, soluble phosphate and minerals in the soil and control or inhibition of the activity of plant pathogens [6-8]. Some PGPR are also responsible for promoting growth indirectly by eliciting induced systemic resistance [9]. Some bacteria such as Klebsiella sp. D5A showed the high plant growth promoting activity on the glycophytic crop in saline-alkaline soils [10,11]. Likewise, many studies have been pulished on beneficial effects of bacterial application on growth of Wheat under salt stress [12-15]. Various bacterial genera like Azospirillium, Arthrobacter, Azotobacter, Azoarcus, Serratia, Bacillus, Pseudomonas, Enterobacter, Rhizobium, Gluconacetobacter, Erwinia, Acinetobacter, Burkholderia, Beijerinckia, and Klebsiella are well known for their PGPR activities [16-19]. These microoragnisms act as promoters of plant growth vi the production of amino acids, Indole Acetic Acid (IAA), gibberellins and other polyamines, improving root growth and, consequently, increasing water and nutrient absorption bt the plants and generating rhizobia-plant interaction sites [20]. Among other benefits, PGPR are also able to solubilize phosphates, produce siderophores, fix N_2 and mitigate biotic and abiotic stresses [21]. In the sense, the co-inoculation of microorganisms with different function can be considered an economically viable and environmentally sustainable strategy to improve plant performance [22,23].

The objective of this study was characterize by different methods includes: morphology, physiology and biochemistry and molecular identification of strain of *Pantoea agglomerans* 20-19, plant growth promoting rhizobacteria in the region Meknes of Morocco.

Materials and Methods

Bacterial strains and growth conditions

The bacterium *Pantoea agglomerans* belongs to the collection of the of Laboratory of Plant Biotechnology and Molecular Biology, Department of Biology, Faculty of Science, University Moulay Ismail (Morocco). This strain was grown at 30°C on LPGA medium (5g/l Yeast extract, 5g/l Peptone, 10g/l Glucose and 18g/l Agar).

Identification of Pantoea 20-19

Genomic DNA was extracted from the bacterial isolate using the Gen Elute Mammalian Genomic miniprep Kit (Sigma-Aldrich, USA). Polymerase chain reaction was used to amplify 500pd fragments using primers Fd1(CAGAGTTTGATCCTGGCTCAG) and RP2 (AGAGTTTGATCCTGGCTCAG). The amplification of 16S rDNA was conducted following the procedure described by El kahkahi et al. 2019 and the amplified DNA products were sequenced at CNRST Laboratory (National Centre for Scientific Research and Technology

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Table 1: Antimicrobial agents and concentrations tested.

Antibiotics	Code	Concentration disc (µg)
Amoxycillin	AML	25
Amoxycillin/Clavulanic acid	AMC	30
Ticarcillin	TIC	75
Imipenem	IMP	10
Cefadroxil	CFR	30
Cefoxitin	FOX	30
Ceftriaxone	CRO	30
Cefixime	CFM	5
Gentamicin	CN	15
Amikacin	AK	30
Colistin	CT	50
Ciprofloxacin	CIP	10
Nadilixid acid	NA	30
Trimethoprim-sulfamethoxazole	SXT	25
Fosfomycin	FOS	50

in Rabat, Morocco).

Morphology, physiology and biochemistry characterization of Pantoea 20-19

Pantoea 20-19 was characterized and tentatively identified on the basis of their biochemical characteristics according to Bergey's manual of determinative bacteriology [24]. These tests were followed by identification using a biochemical API20E (BioMerieux) and ABIS® online software.

Measurement of promoting growth plant activities

Qualitative estimation of phosphate solubilization: The isolate of *Pantoea agglomerans* was screened qualitatively for phosphate solubilization on the PVK medium [25]. The phosphate solubilization ability was analyzed by measure of the diameter of a clear halo zone around colonies after incubation at 30°C for seven days. The phosphate solubilization index (PSI). was calculated by using the following formula [26]. (PSI=[(colony diameter + Halo diameter)/ Colony diameter].

Determination of indole acetic acid production (IAA): IAA production by *Pantoea agglomerans* was estimated with the method of Loper and Scroth [27]. 500µl of bacterial culture (24h old) was inoculated in 50ml of nutrient broth added with 0.1% L-tryptophan and incubated at 30°C for 2 days in the dark. After incubation, the bacterial culture were centrifuged at 10000 rpm for 10min. Salkowski ragent (4ml) was added to one ml of collected supernatant and after 30min incubation pink color developed which indicated production of IAA. To quantify IAA, absorbance was taken at 535nm by using UV/Visible spectrophotometer. The IAA concentration was estimated with a standard curve of IAA.

Determination of ammonia production: The production of ammonia was determined according the Cappucina and Sherman [28]. The bacterial strains were grown in 10ml of peptone water and incubated at 30°C of 2 days. After that, Nessler's reagent (0.5ml) was added in each tube and development of yellow to brown color indicated ammonia production.

Nitrogen fixation: The qualitative estimation of Nitrogen fixation was conducted using the method described by Rajasankar and Ramalingam [29]. A nitrogen free semi-solid medium was used with the following composition: 5g Malic acid, 0.5g K_2HPO_4, 0.2g $MgSO_4$ $7H_2O$, 0.1g NaCl, 0.02g $CaCl_2$, and 0.5% bromothymol blue in 0.2N KOH 2ml, 4ml of 1.64 % Fe-EDTA solution and 2g agar, distilled water (1liter) was then added for a final pH of 7. The cultures were then incubated at 30°C for 5 days and the formation of a pellicle at the sub surface level was considered to be a positive test for N fixation.

Antibiotic sensitivity assay

Antibiotic sensitivity or resistance of *Pantoea agglomerans* was carried out following to the Clinical and Laboratory Standard Institute (CLSI). Fifteen antibiotics (Oxoid) were chosen for the study (Table 1). The sowing on plate was made of each strain on Muller-Hinton agar (MHA) media plates by using swab stick. Antibiotic discs were placed on MHA plates. The plates were incubated at 30°C for 24h. The inhibition zone was measured in millimetres (mm) surrounding the antibiotics discs.

Results

The bacterial isolate 20-19 was isolate from the rhizosphere of Wheat (Triticum aestivum). Microscopic observation revealed that the isolate is a Gram negative rod shaped bacterium. Molecular analysis based on 16S rDNA gene homology identified the 20-19 as *Pantoea agglomerans* with 97% similariy with the reporeted gene sequence (Table 2).

Biochemical tests such as oxidase test, catalase, carbohydrate utilization, citrate utilization etc. were carried out for phenotypic identification of strain. Biochemical characterization of strain and enzymatic activities of the strain were tabulated in Table 3. Briefly, the strain were rod shaped were positive for urea and ONPG. The strain Pantoea agglomerans degraded some carbon sources such as Glucose, Mannitol, Rhamnose and Saccharose. Selected strain was Gram negative rod, catalase positive, oxidase negative, mobile, fermenting glucose without gas production. The strain were characterized by biochemical attributes and were identified as Pantoae agglommerans on the basis of ABIS® online software (Table 3).

Plant growth promoting traits are described in Table 4. *Pantoea agglomerans* is capable of synthesize IAA, ammonia, Nitrogen fixation and phosphorus solubilization.

The antibiotic susceptibility results of *Pantoea agglomerans* is shown in Table 5. *Pantoea agglomerans* was highly sensitive to Amoxycillin, Amoxycillin/Clavulanic acid, Ticarcillin, Imipenem, Cefadroxil, Cefoxitin, Gentamicin, Amikacin, Colistin, Ciprofloxacin, Nadilixid acid and Trimethoprim-sulfamethoxazole. This species showed low resistance to Ceftriaxone, Cefixime and Fosfomycin.

Table 2: Identification of strain 20-19 by 16S rDNA sequencing.

Strain	Number of base pairs	Closest relative sepecies	Similarity (%)	Accession number in Gen Bank (NCBI) of the Strain
20-19	1271	*Pantoea agglomerans*	97%	GQ478021

Table 3: Biochemical characterization of *Panoea agglomerans* 20-19.

	***P. agglomerans* isolate of this study**	**Characteristics of *P. agglomerans Im2* as reported by Silini-Cherif et al. 2012**	**Characteristics of *P. agglomerans* as reported by Loch and Faisal (2007)**
Gram Strain	-	-	-
Production of H_2S	-	-	-
Simmons Citrate	+	+	+
Production of Indole	-	-	-
Voges-Proskauer	-	+	+
Nitrate Reduction	+	+	+
Lysine Decarboxylase	-	-	-
Ornithine Decarboxylase	-	-	-
Arginine Dihydrolase	+	-	-
Catalase	-	+	+
Oxidase	+	-	-
ONPG	-	+	-
Phenylalanine Deaminase	+	-	-
Urease	+	-	-
Motility	+	+	Nd
Acid Production from:			
Glucose	+		
Mannitol	+	+	+
Inositol	-	+	+
Sorbitol	+	Nd	-
Rhamnose	+	Nd	Nd
Saccharose	-	Nd	+
Melibiose	-	Nd	+
Amylose	-	Nd	Nd
Arabinose	-	Nd	Nd

+: Positive test, -: Negative test, ND: Not Determined

Table 4: Plant growth promoting activities by *Pantoea agglomerans* 20-19.

Tests	Phosphorus solubilization	Production of ammonia	Nitrogen fixation	IAA production (µg/ml)
Strain isolated	55%	+	+	156

+: Positive test, -: Negative test

Discussion

The identification of this strain by the ABIS online software gave the specie *Pantoea agglomerans*. The analysis of the 16Sr DNA gene sequence confirmed this identification. On the basis of biochemical test and by comparing the isolated strain *Pantoea agglomerans* 20-19 to biochemical characteristics of type strain *Pantoea agglomerans Im2* [30], two differences revealed: the urea test and Voges-Proskauer. However, the 16S rRNA sequence analysis of *Pantoea agglomerans* 20-19 showed 97% similarity with *Pantoea agglomerans* Im2 type strain GQ 478021 [30] which has been reported as a plant associated bacterium. Hoang and Cao [31], suggested that several genera of Enterobacteriaceae such as *Pantoea agglomerans* were beneficial to plant. These bacteria were able to use a wide variety of cabron sources as nutrients in the rhizosphere [30].

IAA had a positive effect on root system elongation and development which helps in the uptake of water and essential nutrients. This may lead to the increased root growth and develop a healthy plant as compared to control [31-33]. In this study, *Pantoea agglomerans* is able to produce IAA growing in medium addition of triptophan with a value 156 µg/ml. The production of IAA by bacteria isolated from rhizosphere had already been reported in number of studies [30,34-40].

Phosphorus (P) one of the most important nutrients, is frequently available in relatively insoluble forms considered as the limiting nutrient on plant growth and can possibly leading to phosphorus deficiency [41]. In this study, *Pantoea agglomerans* 20-19 showed phosphate solubilization with percentage 55% by the formation of transparent zone around the bacteria colony. Which could be due to synthesis several organic acids (phytases) [16,30,34,35,37,42].

The atmospheric N2 is converted into plantutilizable forms by biological N_2 fixation which changes nitrogen to ammonia by nitrogen fixing microorganisms using a complex enzyme system

Table 5: Overal antimicrobial susceptibility patterns of *Pantoea agglomerans*.

Antibiotics	Diamètre critiques (mm)		Diamètre of zone (mm)	Result
	S≥	R<		
AML	19	19	27	S
AMC	19	19	26	S
TIC	23	20	24	S
IMP	22	17	34	S
CFR	12	12	18	S
FOX	19	15	30	S
CRO	25	20	8	R
CFM	17	17	6	R
CN	17	14	34	S
AK	18	15	32	S
CT	15	15	19	S
CIP	25	22	35	S
NA	14	14	19	S
SXT	14	11	33	S
FOS	14	14	10	R

known as nitrogenase [43]. In our experiments, *Pantoea agglomerans* gave positive result for the nitrogen fixing activity non-nodulating by changing the green color of the medium (Nfb) in a blue color. The utilization of halotolerant PGPR with nitrogen fixation ability is considered a good strategy to improve the growth of salt sensitive plants [44].

Another important PGP trait exhibited by the organism is ammonia production. Accumulation of ammonia in the soil also creates the alkaline conditions which suppresses the growth of certain fungi [45-48]. In this present study *Pantoea agglomerans* exhibted the very good ammonia production activity.

From the above results, it can be concluded that strain *Pantoea agglomerans* can be potentially used as bioinoculants of agricultural in a sustainable way, it requires a long way of greenhouse experiments with pot filled with different type of soils and finally, field experiements to find out the optimun formulations for the inoculums. Thus, the inoculants can perform close to its optimum potential. Future studies concerning commercialization and provisional field applications of integrated stable bio-forumulations as effective biocontrol strategies are in progress.

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