Klebsiella sp. S6; A HALOTOLERANT RHIZOSPHERE BACTERIUM OF *Phragmites communis* L. WITH POTENTIAL PLANT-GROWTH PROMOTION OF PEPPER

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Abstract. *Phragmites communis* L. is a halophytic plant that can withstand harsh environmental conditions such as salinity. In the present study, we isolated a bacterium associated with the rhizosphere of *Phragmites communis* L. Based on morphological and biochemical as well as MALDI-TOF and 16S rRNA sequencing results, the strain S6 was identified as *Klebsiella aerogenes* with a score of 2.34 for MALDI-TOF and a similarity of 99.86% with type strain *Klebsiella aerogenes* KCTC 2190^T. Furthermore, we demonstrated that strain S6 tolerated up to 2.5 M of NaCl and exhibited resistance to only three antibiotics that are frequently used for *Enterobacteriaceae*. Plant growth-promoting (PGP) results showed that the strain S6 had multiple traits, it was able to fix atmospheric nitrogen, solubilized inorganic phosphate ($Ca_3(PO4)_2$ and potassium in addition to the production of siderophores, ammonia, cellulase, protease and amylase. Indole acetic acid (IAA) production was also detected, and the strain S6 showed a positive ACC deaminase activity. Moreover, when the strain S6 was used as an inoculum for pepper seeds, increases in different parameters including shoot and root lengths and fresh and dry weights were shown under normal and saline conditions. We reported in this study, that the inoculation of pepper (*Capsicum annuum* L.) seeds with the *Klebsiella* sp. strain S6 was efficient for pepper growth in normal and salt stress conditions. The results of this study thus indicate that natural plants from saline habitats present a good source for isolating beneficial PGPR to grow crops like pepper under saline conditions.

Key words: *Phragmites communis* L.; *Klebsiella aerogenes*; rhizosphere; arid environments; PGPR; MALDI-TOF; phylogeny.

INTRODUCTION

Plants interact with different microorganisms including several bacterial taxa that can play significant roles in plant growth [17]. Plant-growth-promoting rhizobacteria (PGPR) display many beneficial effects on their host plants as growth promoters and biocontrol agents [30]. Many PGPRs are reported to be isolated from the rhizosphere of different plants and to possess many beneficial attributes [7]. Numerous bacterial strains were used in inoculation studies including diazotrophic bacteria (*Rhizobium* and *Azospirillum*) and phosphate-solubilizing bacteria (*Pseudomonas* and *Bacillus*) [6]. In this context, many published works have documented that a single microbe inoculation can be used for some crops, particularly after using a nonspecific genus like *Klebsiella* [7]. The enhancement of the growth of many crops can be attributed to different mechanisms like nitrogen fixation, inorganic phosphate solubilization, production of siderophores and others [36].

One of the most popular and consumed vegetable crops around the world is pepper (*Capsicum annuum* L.). This plant is susceptible to many biotic aggressors as well as abiotic stresses like salinity [20]. Pepper plants are non-salt-tolerant vegetable and thus when cultivated at high salt levels, we notice a reduction in plant yield causing major economic losses [1]. In this context, the objectives of the present study are: (1) the isolation of a potential PGPR from *Phragmites communis* L. a plant collected from a saline environment, (2) the morphological, biochemical and physiological properties, and antibiotic susceptibility (pathogenicity test) analyses of the isolated strain, (3) the MALDI-TOF and 16S rRNA sequence identification (4) the characterization of the *in vitro* PGPR features of the selected strain (nitrogen fixation, phosphate and potassium solubilization, IAA, ammoniac and siderophores production and ACC deaminase activity), (5) the production of hydrolytic extracellular enzymes and (6) the PGP potency of isolate by seed germination assay *in vivo* on pepper seeds in a pot experiment.

MATERIALS AND METHODS

Sampling and isolation

Phragmites communis L. rhizosphere soil was collected from the M'Zab valley (Ghardaïa) in the Algerian Sahara in a sterile falcon bottle and brought immediately to the laboratory for further processing. Under aseptic conditions, 1 g of sampled rhizospheric soil was suspended in 9 mL of sterilized physiological saline solution (0.85% of NaCl) and then serially diluted to 10^{-7}. From the prepared dilutions, TSA plates (GranuCult™, Merck KGaA Germany) with 0.5 M NaCl were spread and incubated at 30 ± 2°C for 24 h. The strain S6 was picked up from a 10^{-4} dilution plate and was subcultured on the same medium.

Morphological, biochemical and physiological characterization

The selected isolate was characterized morphologically by a macroscopic study of the colony on TSA medium and microscopically after a Gram staining.

Biochemical characterization of the isolate was performed using catalase and oxidase tests. For the catalase test, a few drops of a 3% (v/v) hydrogen peroxide (H_2O_2) solution were added to a fresh culture of the bacterial isolate. The observation of an

effervescent oxygen reaction is an indication of the degradation of H_2O_2 by the catalase enzyme. The wet filter paper method was used to detect the presence of the cytochrome oxidase enzyme. A strip of Whatman filter paper was dipped into a prepared 1% (w/v) solution of the dye tetramethyl-p-phenylenediamine dihydrochloride (TMPD-hydrochloride). The development of an intense purple hue, appearing within a few seconds, indicates the production of oxidase [24]. The biochemical analysis of the bacterial isolate was studied using the API 20E identification kit (API System, France) according to standard laboratory procedure.

Further, the isolate was screened for its potential to tolerate and grow on TSA medium supplemented with NaCl. The isolate was inoculated onto TSA plates containing 0, 0.5, 1, 2, 2.5, 3 and 3.5 M of NaCl. Then, its growth was monitored for 72 h. The results were recorded according to the extent of microbial colony density that appeared on the surface of the agar plates [32].

Identification of the isolate S6

The isolated bacterial strain was identified using a Microflex MALDI-TOF spectrometer (Bruker Daltonics) equipped with a UV laser at a wavelength of 337 nm and flexControl and MBT Copass software (Bruker Daltonics, Bremen, Germany). The isolated strain was subcultured on TSA medium (GranuCult™, Merck KGaA Germany). After incubation, a pure colony was deposited on a 96-point MALDI-TOF MSP target (Bruker Daltonics) using a fine and homogeneous deposition as recommended by the supplier. After drying at room temperature in the chamber, the deposit was then coated with 1 µL of matrix solution (saturated α-cyano-4-hydroxycinnamic acid solution) (Sigma-Aldrich, Canada) prepared according to the supplier's recommendations and dried at room temperature. On each target, the IVD Bacterial Test Standard (BTS) was deposited, as a validation control. After the acquisition of the control and sample mass spectra, the Biotyper software compares them to reference spectra from the database and displays the corresponding identifications [43]. The spectra are evaluated according to specific score values. The identification results were evaluated as follows: if the logarithmic value of the final score is between 2.3 and 3, the isolate is identified to species level; for values between 2 and 2.3, identification is secure to genus level; for values between 1.7 and 2, identification to genus level is likely; for values below 1.7, identification does not work [44].

In addition, molecular identification by 16S rRNA sequence analysis was performed at Macrogen Ltd. (Netherlands). Identification of the nearest phylogenetic neighbors was carried out using the EzTaxon database (http://eztaxon-e.ezbiocloud.net/) [21]. The aligned sequences were used to reconstruct the phylogenetic tree using the neighbor-joining method by MEGA version 11 [33, 41]. The evolutionary distance matrix was generated as described by Jukes and Cantor (1969) [18] and bootstrap analysis was carried out with 1000 replications.

Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed on Mueller Hinton agar plates, using the standard Kirby-Bauer disc diffusion method [10]. A fresh culture of the isolated strain of approximately 0.5 McFarland was plated onto the entire surface of 9 mm Petri dishes. Antibiotic discs (HiMedia hexa) were then aseptically applied to the surface of the solidified agar. The plates were incubated at 30 ± 2°C for 24 h. The test was performed in duplicate and the average zone of inhibition created by each antibiotic was measured and recorded.

Haemolysis testing

To determine the ability of the bacterial isolate to produce haemolysins a fresh culture was plated on blood agar supplemented with 5% (v/v) of rabbit blood. The plate was incubated at 30 ± 2°C for 24 h. The appearance of a clear zone on the plate was interpreted as a positive result [9].

Screening for PGP traits and enzymatic activity

Biological nitrogen fixation

The isolated strain was tested for its ability to fix molecular nitrogen on a solid nitrogen-free (NFb) medium. Approximately, 2 µL of a fresh bacterial suspension was deposited on the culture medium and incubated at 30 ± 2 °C for 48 h. Any growth on this medium reflects the ability of the bacteria to fix nitrogen [5].

Phosphore solubilization

The selected bacterial isolate was tested for its ability to solubilize phosphate using the method described by Nautiyal (1999) [29]. A 2 µL volume of a fresh bacterial suspension was deposited as a spot on the surface of the National Botanical Research Institute's phosphate growth medium (NBRIP) agar plate containing 0.5% tricalcium phosphate as a source of insoluble phosphate. Incubation is done at 30 ± 2 °C for 7 days and the appearance of clear areas around the colonies reflects the solubilization of inorganic phosphates. For quantitative estimation of inorganic phosphate solubilization by the isolate, the method developed by Pikovskaya (1948) [31] was used. After 10 days of incubation at 30 ± 2°C in a liquid Pikovskaya medium, the concentration of the soluble phosphate was estimated from the supernatant by the stannous chloride method [22].

Potassium solubilization

The inorganic potassium solubilization test was carried out on Petri dishes containing Alexandrov medium (Sigma Aldrich, Germany). Approximately, 2 µL of a fresh bacterial suspension was inoculated onto the plate and incubated at 30 ± 2°C for 7 days. Plates that showed a clear zone indicate the ability to solubilize potassium from an insoluble potassium source [40].

Siderophore production

The production of siderophores was demonstrated by the method described by Schwyn and Neilands (1987) [35] using the medium Chrome Azurol Sulphonate (CAS). This culture medium initially has a blue colour due to the iron-CAS hexadecyl trimethyl ammonium complex (Iron/CAS/HDTMA complex). On solid CAS medium, a 2 µL spot of the bacterial culture was plated and incubated at 30 ± 2°C for 48 h. A colour change of the medium around the colony to yellow-orange confirms the production of siderophores by the bacteria.

Ammonia production

Ammonia (NH_3) production was measured on peptone water by the method described by Cappuccino and Sherman (1998) [8]. A volume of 100 µL of a 24 h fresh bacterial suspension was inoculated into 10 mL of peptone water and incubated at 30 ± 2ºC for 48 h. The addition of 0.5 mL of Nessler's reagent giving a yellow to brown colour indicates NH_3 production. Optical density was measured at 450 nm using the spectrophotometer. The concentration of ammonia was estimated using the standard curve of ammonium sulfate.

ACC deaminase activity

The existence of ACC deaminase activity was determined by the ability of bacterial strains to use ACC as the unique nitrogen source. Using the modified method of Glick et al. (1995) [12], the bacterial strain was tested for its ability to degrade 1-aminocyclopropane-1-carboxylate (ACC) on a minimal medium of DF salts containing 3 mM ACC as the unique nitrogen source. The strain was grown in the presence of two nitrogen sources, ACC and ammonium sulphate $(NH_4)_2SO_4$ and a mineral source magnesium sulphate ($MgSO_4 \times 7H_2O$). The plates were incubated at 30 ± 2°C for 48 h. ACC deaminase activity was positive for the strain with a higher growth rate compared to the medium with $MgSO_4 \times 7H_2O$, indicating the use of ACC as a carbon source.

Indolic compound synthesis

The ability of the studied strain to produce indole acetic acid (IAA) was tested by the colorimetric method described by Gordon and Weber (1951) [14]. A volume of 100 µL of fresh culture was inoculated into 100 mL of Tryptic Soy Broth (TSB, 1:10 dilution) medium, with the addition or not of L-tryptophan (500 $\mu g\ mL^{-1}$). After incubation at 30 ± 2°C for 4 days in the dark with continuous shaking at 180 rpm, the cultures were centrifuged at 10000 rpm at 4°C for 20 min and the supernatant was filtered. To 1 mL of filtrate, 2 mL of Salkowski's reagent (50 mL of 35% hydrochloric acid and 1 mL of 0.5 M $FeCl_3$) and 2 µL of ortho-phosphoric acid were added. The optical density was measured at 530 nm. IAA concentrations were determined using a calibration curve obtained in the range of 0 to 10^{-5} M IAA (Sigma Aldrich, Germany).

Screening for the production of extracellular enzymes

Strain S6 was evaluated for its production of extracellular enzymes namely amylase, cellulase and protease. The hydrolytic activity of the starch was revealed by a test on nutrient agar enriched with 0.5% of soluble starch. Two microliters of a fresh suspension of the isolate were spotted onto the Petri dish containing the prepared culture medium and incubated at 30 ± 2°C for 48 h [2]. After incubation, a reagent dye solution (25.4 mg iodine and 400 mg potassium iodide in 100 mL) was poured onto the surface of the medium and the appearance of a clear zone around the discs indicates the presence of amylase activity.

To study cellulase activity, we used the method described by Teather and Wood (1982) [42]. A basic agar medium that contains 10g/L Carboxy Methyl Cellulose (CMC) was used as the unique carbon source. The plates were inoculated with a fresh bacterial suspension and incubated at 30 ± 2°C for 8 days. At the end of the incubation period, an aqueous solution of Congo red (1%) was added to the surface of the colonies, after 30 min, the surface is flooded with a 1M NaCl solution. Cellulase activity is shown by the appearance of a clear halo around the colonies.

Protease activity was revealed on Skim Milk Agar medium. Spots of 2 µL of a fresh suspension of the isolated bacterial strain were placed on a dish containing the medium and incubated at 30 ± 2°C for 48 h. The appearance of a transparent halo around the spots reflects the presence of hydrolytic activity [4].

***In vivo* evaluation for growth promotion of pepper**

After cleaning in sterile distilled water, the pepper seeds were surface disinfected with a 70% ethanol solution for 1 min, then in 1% sodium hypochlorite for 10 min. After this disinfection, the seeds were washed aseptically with sterile distilled water and then dried [16].

The selected bacterial strain was grown at 30 ± 2°C under agitation at 150 rpm for 24 h on Tryptic Soy Broth (TSB). Afterwards, the culture was centrifuged (10000 rpm/15 min), rinsed twice in PBS and adjusted to a density of about 10^8 bacteria/mL [25]. Inoculation is done after immersion of the seeds in the bacterial suspension for 30 min. The non-inoculated seeds (control) were immersed in sterile distilled water.

For the pot experiment, plastic pots disinfected with ethanol 70° on the inner surface were filled with 150 g of an equal mixture of peat/perlite/sand. The mixture was pre-sterilized in an autoclave at 120°C for 20 min, for three successive days [19]. The pots were then divided into two groups according to the concentration of NaCl used (0 mM and 150 mM). The treated seeds are sown at a rate of 5 seeds per pot at a depth of 1 cm from the surface. The experiment was performed in triplicate for 30 days in an automated greenhouse with an average day/night temperature of 30°C and 16°C respectively and a 16 h photoperiod of sunlight. Soil moisture was adjusted and kept constant during the experiment by watering with sterile distilled water. The

following parameters: stem length, root length, fresh weight, dry weight, leaf area and chlorophyll measurements were recorded after 30 days. For the determination of the chlorophyll ($\mu g/cm^2$) content, a portable chlorophyll meter Konica Minolta SPAD 502 Plus was used.

Data analysis

The data were presented as Mean ± Standard Deviation (SD) of triplicate for experiments. Analysis of variance followed by post-hoc Tukey test ($p < 0.05$) were used to compare treatment means. All the statistical analyses were carried out using SPSS 10.0.

RESULTS

Isolation and identification of strain S6

The isolated bacterium S6 had an irregular form, brilliancy and beige color of the colony on TSA medium supplemented with 0.5 M of NaCl. This strain demonstrated good growth on TSA media supplemented with 0.5 to 2.5 M of NaCl and showed a Gram-negative staining, oxidase-negative, catalase-positive, negative reactions for indole, methyl red, urease, Hydrogen sulphide (H_2S), arginine dihydrolase and gelatin production, and positive reactions for Simmons citrate, Voges-Proskauer (VP), lysine decarboxylase, ornithine and glucose, maltose, saccharose and lactose fermentation tests (Table 1).

The findings demonstrated that the MALDI-TOF analysis provided a score of 2.34 with the closely related strain *Klebsiella aerogenes* (Table 2). The 16S rRNA gene sequence of strain S6 was deposited in GenBank under the accession number OM326713. The alignment of strain S6 with those of reference species available in the EzTaxon-e server can be seen in the obtained neighbor-joining tree (Figure 1). The results of the 16S rRNA sequencing confirmed those of MALDI-TOF with the phylogenetic analysis revealing that strain S6 is 99.86% similar to the type strain *Klebsiella aerogenes* KCTC 2190^T.

Antibiotic susceptibility testing

As shown in Table 3, strain S6 was susceptible to amoxicillin (AMX) (100%), ampicillin (AM) and cefoxitin (FOX). Furthermore, a high rate of resistance was recorded for the following antibiotics: cefalotin (CF), cefotaxime (CTX), imipenem (IMP), nalidixic acid (NA), ciprofloxacin (CIP), amikacin (AN), gentamycin (GN), colistin (CS), cotrimoxazole (SXT), chloramphenicol (C) and fusidic acid (FA).

Table 1. Morphological, biochemical and physiological description of *Klebsiella* sp. strains S6

Test	Strain S6
Gram reaction	N
Colony form	Irregular
Colony color	Beige on TSA medium
Oxidase	-
Catalase	+
Gelatin hydrolysis	-
Urease	-
Nitrate reduction	+
Arginine dihydrolase	-
Indole production	-
H_2S	-
Lysine decarboxylase	+
Simmons citrate	+
VP	+
Methyl red	-
Ornithine	+
Arabinose	-
Amygdalin	-
Melbiose	-
Sucrose	-
Rhamnose	-
Sorbitol	-
Inositol	-
Maltose	+
Saccharose	+
Lactose	+
Glucose	+
NaCl tolerance	0-2.5 M

+ Indicates a positive reaction while - indicates a negative reaction.
N: negative Gram reaction, TSA: Tryptic Soy Agar.

Table 2. MALDI-TOF and 16S rRNA sequence identification of *Klebsiella* sp. strain S6

Strain code	MALDI-TOF species match	Score	GenBank accession number	Sequence length (pb)	Closest relative by EZ Taxon	% sequence similarity
S6	*Klebsiella aerogenes*	2.34	OM326713	1464	*Klebsiella aerogenes* KCTC 2190^T	99.86

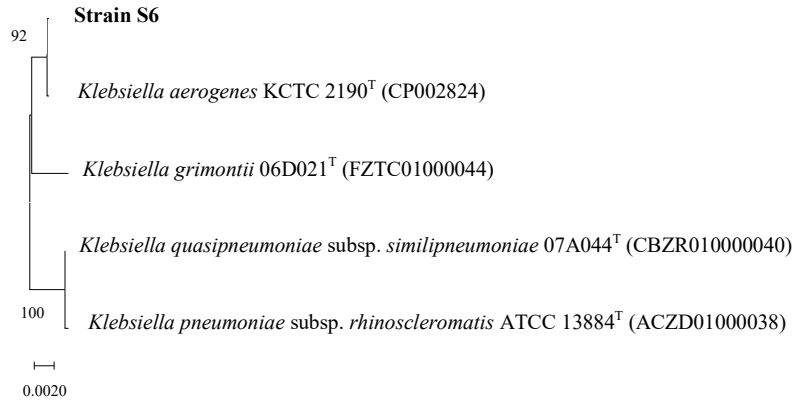

Figure 1. Tree shows the phylogenetic affiliation of strain S6 partial 16S rRNA sequences, a rhizospheric halotolerant bacterium isolated from *Phragmites communis* L. The scale bar shows the number of nucleotide substitutions per site. The evolutionary history was inferred using the Neighbor-Joining method of Saitou and Nei (1987) [33]. The optimal tree is shown. Evolutionary analyses were conducted in MEGA11 [41].

Table 3. Antibiotic resistance profiles (disc diffusion testing) of *Klebsiella* sp. strain S6

Antibiotic family	Antibiotic	Strain S6
Beta-lactam	AMX	R
	AM	R
	CF	S
	FOX	R
	CTX	S
	IMP	S
Quinolone	NA	S
	CIP	S
Aminoside	AN	S
	GN	S
Polymyxin	CS	S
Trimethoprim/Sulfamethoxazole	SXT	S
Chloramphenicol	C	S
Fusidanin	FA	S

AM: Ampicillin, AMX: Amoxicillin, CF: Cefalotin, FOX: Cefoxitin, CTX: Cefotaxime, IMP: Imipenem, GN: Gentamicin, AN: Amikacin, CIP: Ciprofloxacin, SXT: Trimethoprim/Sulfamethoxazole, CS: Colistin, NA: Nalidixic Acid, FA: Fusidic Acid, C: Chloramphenicol. S: Sensitive, R: Resistant.

Plant growth promotion features

The results of the PGP traits of the halotolerant bacterium S6 are given in Table 4. All the tested traits were positive for this strain including Nitrogen fixation, phosphorus solubilization in solid NBRIP medium and liquid Pikovskaya medium (31 µg/mL), potassium solubilization, siderophores, ammonia (42.01 µg/mL) and IAA (101.7 µg/mL) production and a positive ACC deaminase activity.

Production of lytic enzymes

Screening for hydrolytic enzymes activity showed that the strain S6 was positive for protease, cellulase and amylase as shown in Table 4.

Pot experiment

As the rhizospheric strain S6 showed both notable salt tolerance *in vitro* and ACC-deaminase activity, it was further selected for salt stress control in pepper plants. A pot experiment was designed, and the analysis of the results after 30 days of growth under controlled greenhouse conditions is shown in Table 5 and Figure 2.

An increase in stem and root lengths, fresh and dry weights, leaf area as well as chlorophyll measurement were noticed between the control and the inoculated groups under non-saline conditions. For the salt-treated groups, a significant amelioration of the measured parameters was also observed between the control and the inoculated pots with strain S6. Overall, the inoculation with strain S6 was beneficial under normal and saline conditions after comparison to the uninoculated pepper seeds.

Table 4. Plant growth-promoting traits and lytic enzymes activities of *Klebsiella* sp. strain S6

PGP characteristics								Hydrolytic enzyme production		
N_2 fixation	P-solubilization in solid NBRIP medium	P-solubilization in liquid Pikovskaya medium (µg/mL)	K-solubilization	Siderophores	ACC deaminase	NH_3 production (µg/mL)	IAA production (µg/mL)	Amylase	Cellulase	Protease
+	+	31	+	+	+	42.01	101.7	++	+	+++

Production of siderophores and ammonia shows the intensity of orange/pink color halo or yellow/brown color, respectively (+ weak, ++ medium and +++ strong) on blue agar medium CAS and peptone water broth. The remaining tests were classified as positive (+) or negative (-).

Table 5. Effects of PGP of *Klebsiella* sp. strain S6 on the stem and root lengths, fresh and dry weights, leaf area and chlorophyll measurement of pepper (*Capsicum annuum* L.) seedlings cultivated under standard conditions assay of greenhouse irrigated without (0 mM NaCl) and with saline (150 Mm NaCl) solution

Growth parameter	0 mM NaCl		150 mM NaCl	
	C1	S6	C2	S6
Stem length (cm)	5.6 ± 0.5^{a}	13.34 ± 1.06^{b}	4.48 ± 0.75^{a}	9.70 ± 1.53^{c}
Root length (cm)	4.78 ± 0.52^{a}	11.25 ± 1.17^{b}	2.98 ± 0.42^{a}	8.13 ± 1.21^{c}
Fresh weight (g)	4.49 ± 0.41^{a}	9.31 ± 1.35^{b}	2.61 ± 0.41^{a}	6.68 ± 1.45^{c}
Dry weight (g)	0.51 ± 0.076^{a}	0.88 ± 0.08^{b}	0.29 ± 0.06^{ac}	0.73 ± 0.2^{ab}
Leaf area (cm^2)	48.18 ± 7.90^{a}	85.10 ± 6.23^{b}	25.55 ± 6.32^{c}	53.40 ± 6.19^{a}
Chlorophyll measurement (SPAD values)	40.32 ± 1.05^{a}	38.07 ± 0.90^{a}	43.15 ± 1.53^{b}	40.18 ± 0.75^{a}

C: Control (non-inoculated), S6: Treated seeds (inoculated). Data is the mean of 24 plants obtained from six replicates. Different letters within each column mean values are significantly different using Tukey test ($p \leq 0.05$).

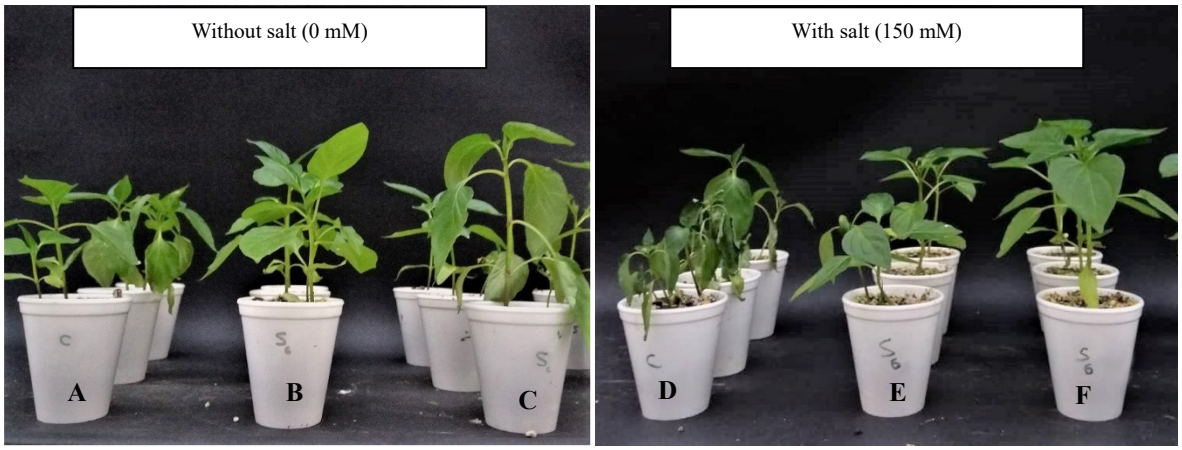

Figure 2. Morphological appearance of pepper plants 30 days after sowing. Uninoculated plants (A: under normal condition and D: under salt condition), inoculated plants with S6 under normal condition (0 mM) (B and C) and salt condition (150 mM) (D and E)

DISCUSSION

Different methods are used in order to increase plant tolerance to salt stress. These methods include the use of salt-tolerant crops, traditional breeding, transgenic plant genetic engineering as well as the PGPB as a more sustainable approach [3]. These latter are highly abundant in the rhizosphere and the endophytic compartment of plants [26, 39].

One of the plants that are the subject of studying its associated rhizospheric bacteria is *Phragmites communis* L. It is a halophytic plant that belongs to the genus *Phragmites* of the *Poaceae* family is widely distributed in arid and semi-arid regions and is known for its growth under harsh saline conditions [23]. Thus, this makes this plant a good candidate for searching for associated bacteria that can promote plant growth under plant-limiting environments as the case for saline conditions. Similar studies were conducted on different plants. For example, the study of *S. fruticosa* from the saline desert of Little Rann of Kutch, Gujarat (India) revealed that Bacillus licheniformis A2 had a positive growth promotion of Groundnut (*Arachis hypogaea*) under salt stress [15]. Further, a strain named *Arthrobacter pascens* that was isolated from the rhizosphere of the same plant collected from high saline fields of District Mardan in Pakistan showed reliability in the growth promotion of maize crop in all the physiological parameters [45].

The different bacterial taxa harbored in the rhizosphere can directly or indirectly influence the plant growth by different mechanisms including atmospheric nitrogen fixation, solubilization of various minerals such as phosphorus and potassium, production of siderophores and the possession of an ACC deaminase activity can impact the plants when facing salt stress [26].

Many researchers have been working on finding PGP bacteria associated with different plant species and searching for their application in agriculture [39]. Different genera like *Pseudomonas*, *Bacillus*, *Pantoea* and *Klebsiella* were found in the rhizosphere of different plant species with interesting biological activities [11, 27].

In the present study, we identified a rhizospheric bacterium strain S6 from *Phragmites communis* L. rhizosphere, a plant able to grow in harsh environmental conditions. The different performed tests (morphological, biochemical and molecular identification) led to the identification of our strain S6 as *Klebsiella aerogenes*. This strain demonstrated a good growth on TSA medium supplemented with up to 2.5 M of NaCl concentration.

Screening for *in vitro* PGP properties demonstrated that the majority of the tests were positive. the strain was capable to produce IAA, ammonia, siderophores, ACC deaminase and fixing atmospheric nitrogen as well as solubilizing phosphorus and potassium. IAA is a phytohormone that regulates many physiological aspects of the plants as it is responsible for improving root elongation [16]. Moreover, ammonia production by bacterial strains is reported to promote root and shoot elongation of plants [28]. Furthermore, siderophores are low-weight molecular iron-chelating molecules that act as scavengers making iron unavailable for phytopathogens and directly facilitating iron uptake from the soil by plants [36]. On the other hand, the ACC-deaminase activity was positive indicating that the strain S6 is able to help in plant growth and maintenance under saline conditions [13]. This activity of ACC deaminase has been reported in the literature to protect plants from the harmful effects of stress-induced ethylene by cleaving ACC [12, 13].
In fact, ACC is the direct precursor of ethylene in all higher plants. Thus, PGPR that contains ACC deaminase activity can degrade ACC, resulting in the production of α-ketobutyrate and ammonia and thereby decreasing the production of plant growth-inhibitory ethylene [31]. Furthermore, ethylene is involved in different aspects of plant growth like seed germination, root hair development, root elongation, fruit ripening, organ senescence ... etc. Nevertheless, different biotic and abiotic stresses can cause an imbalance in ethylene production and an increased level of ethylene can

inhibit root and shoot length and overall plant growth [37].

In addition, nitrogen, potassium and phosphorus are limiting nutrients for crop yields, growth, and development. Bacteria possessing these abilities can be major contributors to the conversion of atmospheric nitrogen to ammonia and solubilization of phosphorus and potassium in soils that ultimately help in the promotion of plant growth [47].

For salt-stress mitigation assay in planta, since inoculation with ACC deaminase producing PGPB is frequently recommended with regard to its significant potential role in the reduction of stress-induced ethylene defense pathway, six of the halotolerant isolates that exhibited ACC deaminase activity were evaluated for their tomato growth promotion ability [37, 47]. Under greenhouse conditions at 0 and 150 mM of NaCl, inoculation of pepper seeds by strain S6 gave a noticeable increase in the majority of the recorded parameters in both inoculated groups with and without NaCl addition. As reported previously, pepper plants are sensitive to salt stress [1]. The use of rhizospheric bacteria on pepper plants was highlighted in the study of Wang et al. (2018) [47] where 3 strains of *Bacillus* out of 13 strains isolated from saline soils from Xinjiang in China had a positive activity of ACC deaminase showed a significant increase in fresh and dry weight, the length of the roots and the aerial portions of pepper under salt stress. Furthermore, Siddikee et al. (2011) [37] showed in their study that the use of ACC deaminase-producing halotolerant bacteria mitigates the salt stress by reducing salt stress-induced ethylene production in the growth of red pepper plants. *Klebsiella* strains with aminocyclopropane-1-carboxylate deaminase (ACCD) activity have been reported by many authors to protect plants from the deleterious effects of abiotic stressors like the case of salinity [24, 34, 38].

We noticed a notable increase in leaf area under normal and saline conditions. Thus, the higher biomass accumulation could be due to the greater photosynthetic area and the increased CO_2 availability through the open stomata [46]. In our experiment, there was no significant modification of leaf color due to bacterial inoculum that can indicate changes in leaf pigment. Thus, our tested PGPR did not increase the accumulation of chlorophyll.

The present study reports the role of the multiple plant-growth-promoting bacterium *Klebsiella* sp. strain S6 in improving the growth of pepper under normal and salt stress conditions. The strain S6 that was isolated from the rhizosphere of *Phragmites communis* L. growing in the desert region of Ghardaïa (Algeria) would be a good candidate for an evaluation in the field under a variety of agronomic trial conditions.

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