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Free-Living Diazotrophic Rhizobacteria with Plant Growth Promoting Traits Isolated from the Rhizosphere of Wheat Grown on Saline Soil in West Algeria

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ABSTRACT

Plant growth promoting rhizobacteria (PGPR) represent a very small portion of plant rhizobacteria that have the ability to promote plants growth. In this work we evaluated the potential of free-living diazotrophic bacteria isolated from saline soil in west Algeria for the presence of PGP traits. Selective media were used for the isolation and selection of these plant growth-promoting rhizobacteria. A total of 296 strains were isolated from rhizospheres of wheat in eleven different saline soil areas of the perimeter of Mina (Relizane province – west Algeria). All the strains were evaluated for Indole acetic acid (IAA) production, phosphate solubilization, siderophores production, Hydrogen cyanide (HCN) production and ammonia production. Amongst these 296 strains 35 with multiple PGPR traits were in addition tested for their ability to use ACC as a sole nitrogen source and for nitrogenase activity using the Acethylene Reduction Activity test. These thirty five strains selected for their high PGP traits performance were further characterized by 16S rRNA sequencing.
KEY WORDS: diazotrophic bacteria, PGPR, IAA, ACC, HCN, Siderophore

INTRODUCTION

Beneficial soil microorganisms such as PGPRs have received attention of researchers all over the world because of their potential use to develop a durable agriculture. They represent a very small portion of rhizobacteria (plant root associated bacteria) that have the ability to promote growth of plants and protecting them from disease and abiotic stresses [1, 2]. The PGPR promote plant growth by several mechanisms which they are not completely understood, but they supposed to include (i) the production of plant growth regulators like phytohormones [3], (ii) atmospheric nitrogen fixation [4], (iii) phosphate solubilization [5] and (iv) antagonism against phytopathogenic microorganisms [6].

The soil salinity presents major challenge in agriculture for many countries. To solve this problem, many strategies can be used including the application of halotolerant plant growth promoting Rhizobacteria to improve plant cultivation in saline soils [7].In microbial selection salinity soil salinity plays an important role as environmental stress which is known as a reducing factor of bacterial diversity [8]

A few studies of researchers were focusing on salt tolerant Rhizobacteria such as those reported by [9] but the information on them are still limited [10]. Because soil salinity grows in many parts of the world due to irrigation, there is a need to improve the performance of the plant and biological nitrogen fixation (BNF) in salt stress conditions [11].

In addition to having PGP traits, PGPR strains must be rhizospheric competent, able to survive and colonize the plant [12].

This study was designed to explore wheat rhizosphere microbial diversity of diazotrophic bacteria having combinations of PGP activities and belonging to *Klebsiella*, *Stenotrophomonas*, *Serratia*, *Pseudomonas* and *Bacillus* genus for their multiple PGP traits.

METHODS

Collection of Soil Samples:

Soil samples were collected from different saline soil areas in the perimeter of Mina (Relizane province – west Algeria, (35°44′N 0°33′E) between April 2014 and March 2015. The samples were brought to laboratory and pH, soil moisture and electrical conductivity were measured.

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Isolation of diazotrophic bacteria:

For the isolation of diazotrophic bacteria, 3 different N-poor medium were used: (i): N free semi solid malate medium [13], (ii): Burk medium [14] and (iii): Ashby medium [15]. The pure culture obtained were maintained on nutriment agar slants and stored at 4°C for subsequent analysis.

The preliminary identification was carried out on the bases of cellular morphology, Gram stain, colony morphology, and biochemical characterization.

In vitro screening of bacterial isolates for their Nitrogen fixation capacity:

The acetylene reduction assay was used to determine The Nitrogenase activity. All the isolates were grown on N free semi solid medium in 20 mL sterile vials containing 12 mL of medium. Cultures were grown at 30°C without shaking. After 4 days of incubation, 500 µl of acetylene was injected, and the incubation was continued for 14h. The presence of ethylene was detected by gas chromatography in Agilent Technologies 7820A chromatograph as described in [16].

Assay for Indole acetic acid (IAA) production:

Quantification of IAA production was performed using the method of [17]. Bacterial cultures were grown for 72 h with 160 rpm on a rotary shaker on their respective media supplemented with 1 g/l of NH_4Cl and 200 mg/l of tryptophan at 30° C. Cultures were centrifuged at 6000 rpm for 20 min. 1 ml of the supernatant was mixed with 2ml of the Salkowski reagent (50 ml, 35% of perchloric acid, 1ml 0.5M $FeCl_3$ solution) and two drops of orthophosphoric acid. Development of a pink color indicates IAA production. Optical density was taken at 530 ηm using an UV-visible spectrophotometer (JENWAY 6715). The amount of IAA produced by cultures was estimated against a calibration curve of IAA obtained in the range of 0–100 mg/ml.

Screening of phosphate solubilizing ability:

Qualitative estimation of phosphate solubilization:

The capacity of bacterial isolates to solubilize phosphate was evaluated qualitatively using Pikovskaya's agar plates [18]. All strains were inoculated and incubated at 30° C for 7 days. Appearance of a clear halo surrounding the developed bacterial colonies was considered as positive for phosphate solubilization.

Quantitative estimation of phosphate solubilization:

Quantitative assay of phosphate solubilization was done in liquid culture using National Botanical Research Institute's phosphate growth medium (NBRIP) [19]. Strains were inoculated into 50ml of NBRIP medium in 250ml Erlenmeyer flask, incubated at 30° C with shaking at 160 rpm for 7 days, then centrifuged at 6000 × *g* for 20 min. The content of soluble Phosphate in the supernatant was measured by the Vanado-molybdate-yellow colorimetric Method [20]. The phosphorus content was estimated against a calibration curve of standard KH_2PO4 solution.

Siderophore production:

All the isolates were tested for the production of siderophores using chrome azurol S agar (CAS) described by [21]. plates containing CAS agar were prepared and inoculated with test strain. After 4 to 5 days of incubation at 30 ° C, the change of Blue color to yellow-orange around the colony is considered as positive result for siderophore production

In vitro ACC-metabolism assay:

Selection of isolates for ACC metabolism was done based on the ability of isolates to use ACC as a sole nitrogen source. The ACC metabolism assay was carried out using to the method described by [22]. All isolates were inoculated into tube containing 5ml of TSB for 2 days at 30° C with shaking at 160 rpm. Then the cultures were diluted ten times in sterile 0.1 M of $MgS0_4$.In 96-well plate, 150 µL of DF salts medium [23] was added in all wells. In lane 3, 6, 9 and 12, 15 µL 0.1M $MgS0_4$ was added and in lane 2, 5, 8, and 11, 15µL 0.1 M NH_4S0_4 was added. 3 mM ACC was filter sterilized with 0.2µm syringe filter and was stored at -20°C before the assay. This was allowed to thaw before use; 15µL of ACC was filled in the lane 1, 4, 7 and 10. In each well 22µL of bacterial culture were used for inoculation and 22µL 0.1M $MgS0_4$ was used for control wells. Well plate reader Labsystems®) was used to measure the optical density after 0, 24, 48, 72 and 96 hours at 620 ηm.

Hydrogen cyanide production

All bacterial isolates were tested for their ability to produce HCN. Using the method of [24], Nutrient broth was modified with 4.4 g / l of glycine and bacteria were streaked on them. A filter paper soaked in sodium carbonate 2% in a picric acid solution 0.5% was placed in the upper part of the plate. The plates were sealed with parafilm M® and incubated at 30 ° C for 96h. Orange to red developing color from initial yellow indicates HCN production.

Ammonia production:

All isolates were tested for ammonia production in peptone water. Bacterial cultures were inoculated into tube containing 10 ml of peptone water and incubated for 2-3 days at 30° C. In each tube 0.5 ml of Nessler's reagent was added. brown to yellow color development was a positive test for ammonia production. [25]

Genotypic identification of isolates

16S rRNA gene partial sequencing of different isolates was used to identify bacterial isolates. For the extraction of total DNA, NucleoSpin® Microbial DNA (Macherey Nagel GmbH & Co.KG . Neumann-Neander-Str.6-8. 52355 Dűren.Germany, http://www.mn-net.com/) was used. For the amplification of the 16S rRNA gene from genomic DNA by means of polymerase chain reaction, universal Forward Primer (1390): 5_-AACGGGCGGTGTGTRCAA-3 and Reverse Primer (PA2): 5_- AGTTTGATCMTGGCTCAG-3 were used [26]. The sequencing of PCR products of partial 16S rDNA genes were done by Eurofins Genomics GmbH (http://www.eurofinsgenomics.eu/). BLASTn program (NCBI BLAST® homepage: http://blast.ncbi.nlm.nih.gov) was utilized to analyze the sequence. All sequences of 16S rRNA gene were aligned by ClustalW using MEGA 6.0 software (http://www.megasoftware.net/) and a neighbor-joining (NJ) tree was generated using the same software.

Statistical analysis:

All the experiments were performed in triplicate. The data were evaluated by analysis of variance using Statbox version 6.4 (http://www.statbox.com/). The comparison of means was performed using the least significant difference (LSD) within confidence limits of $p \leq 0,05$.

RESULTS

Eleven soil samples were collected in the region of Mina (Relizane province – west Algeria). The GPS coordinates of the sampling area are given Table 1. Soil samples were transported to the laboratory and the physical properties were measured (Table.1). In general the pH was alkaline with values ranging from 7,67 to 8,12. The soil moisture ranged from 11,33 % to 17,84% and the electrical conductivity values ranged from 6,10 to 13,30 dm/S.

Table 1. pH, soil moisture and electrical conductivity of soil samples

Sample	coordinated map	electrical Conductivity dm/S	pH	Moisture %
1	0° 29` 24 `` E 35° 44` 23`` N	9,60 ± 0,14	7,72 ±0,04	15,88 ±0,12
2	0° 29` 32 `` E 35° 44` 14`` N	9,10 ± 0,16	7,95 ±0,07	15,86 ±0,22
3	0° 29` 15 `` E 35° 44` 09`` N	9,90 ± 0,06	8,00 ±0,03	17,40 ±0,16
4	0° 42` 10`` E 35° 57` 15`` N	9,60 ± 0,23	7,67 ±0,06	14,44 ±0,34
5	0° 42` 32`` E 35° 57` 09`` N	8,00 ± 0,11	7,89 ±0,09	13,39 ±0,24
6	0° 44` 34`` E 35° 56` 48`` N	12,50 ± 0,22	7,98 ±0,04	12,34 ±0,32
7	0° 37` 16`` E 35° 55` 19`` N	13,30 ± 0,09	7,97 ±0,08	13,66 ±0,44
8	0° 36` 59`` E 35° 55` 24`` N	8,40 ± 0,11	7,71 ±0,08	13,03 ±0,38
9	0° 41` 02`` E 35° 57` 30`` N	7,30 ± 0,13	7,79 ±0,02	12,35 ±0,21
10	0° 44` 45`` E 35° 56` 00`` N	6,40 ± 0,16	7,98 ±0,06	11,92 ±0,18
11	0° 38` 20`` E 35° 55` 23`` N	6,30 ± 0,11	7,93 ±0,04	11,33 ±0,27
12	0° 36` 50 `` E 35° 55` 00`` N	8,80 ± 0,10	7,71 ±0,01	11,39 ±0,17
13	0° 41` 01`` E 35° 56` 33`` N	6,90 ± 0,13	7,80 ±0,02	13,79 ±0,24
14	0° 44` 42`` E 35° 55` 43`` N	8,00 ± 0,08	7,79 ±0,04	13,92 ±0,08
15	0° 29` 17 `` E 35° 43` 54`` N	6,10 ± 0,09	8,12 ±0,05	17,84 ±0,31

Numerical values are mean±SD of three independent observations, values with different letters are significantly different at P<0.05 in all the treatments.

Nitrogen fixation capacity of the isolated strains

Selective nitrogen poor media were used for the isolation and selection of diazotrophic growth-promoting rhizobacteria. We selected a total of 296 isolates amongst the growing colonies that were subsequently screened for their multiple PGP traits. From the 296 isolates (Data not shown) only 35 were retained for their high-performance PGP traits (Table 2) and are described below.

In order to know if the bacteria isolated on nitrogen poor media are effective diazotrophs we measured their nitrogen fixation capacity using the Acetylene Reduction Assay as described in Material and Methods. Four strains (NHA13, NHA79, NHA66, NHB9) showed acetylene reduction activities ranging from 3,29±0,13 to 2,19±0,01^{c} ηmol C_2H_4 /h and represent the most efficient diazotrophs in this assay. Twenty four strains had intermediate values between 1 ηmol C_2H_4 /h and 0,05 ηmol C_2H_4 /h. The other seven isolates were below 0,05 ηmol C_2H_4 /h or had no activity at all (NHA67) (Table 2).

Table 2. Summary of plant growth promoting traits showed by diazotrophic isolates

Isolat	ARA ηmolC_2H_4/h	IAA	Phosphate	ACC de-aminase	Siderophore	HCN	Ammonia
NHA13	3,29±0,13^{a}	5,05±0,83^{u}	185,91±5,72^{o}	++	+	-	+
MHA24	0,03±0,00^{s}	13,67±0,28^{o}	337,91±5,24^{j}	+	+++	-	+
BHA10	0,85±0,04^{f}	5,62±0,47^{stu}	557,52±16,43^{d}	-	+	-	+
BHA0	0,57±0,02^{ij}	7,35±0,76^{r}	47,67±5,51^{r}	-	+	-	+
NHA19	0,70±0,01^{h}	6,84±0,27^{rs}	568,19±4,97^{d}	+++	++	-	+
NHA79	2,22±0,01^{c}	74,81±0,94^{a}	145,81±3,02^{p}	++	+++	-	+
NMB8	0,60±0,01^{ij}	67,37±0,13^{c}	183,71±3,74^{o}	-	++	-	+
NHA67	0,00±0,00^{s}	63,76±0,21^{d}	402,43±5,01^{h}	-	+	-	+
BHA62	1,05±0,00^{e}	5,43±0,41^{tv}	262,43±5,76^{m}	-	+	-	+
SHB91	0,35±0,03^{mn}	47,23±0,74^{e}	656,81±16,72^{bc}	-	++	-	+
SHB54	0,48±0,01^{k}	15,78±0,57^{n}	118,71±11,54^{q}	-	+	-	+
MHB62	0,32±0,01^{mno}	23,25±0,58^{i}	98,71±6,73^{q}	++	+	-	+
MHC9	0,31±0,02^{no}	14,66±0,57^{no}	101,10±2,86^{q}	-	-	-	+
MHC54	0,29±0,01^{o}	72,62±0,48^{b}	291,29±5,91^{l}	-	+	-	+
MHB52	0,36±0,01^{mn}	13,52±0,54^{o}	527,91±7,09^{e}	+	+	-	+
SHB57	0,54±0,02^{j}	18,48±0,56^{l}	299,00±57,26^{kl}	-	-	-	+
NHA21	0,27±0,01^{o}	6,69±1,04^{rst}	672,33±7,37^{bc}	+++	+++	-	+
SHA21	0,76±0,01^{g}	7,79±0,37^{r}	579,57±9,50^{d}	++	+++	+	+
NHA15	0,01±0,00^{s}	25,36±0,57^{h}	422,00±8,54^{h}	+	+	-	+
MHA59	0,22±0,00^{p}	25,90±0,44^{gh}	447,95±5,58^{g}	-	+	-	+
MHB56	0,56±0,01^{ij}	41,59±0,52^{f}	320,91±1,15^{jk}	-	++	-	+
SMA4	0,03±0,00^{s}	5,69±0,14^{stu}	646,71±6,60^{c}	-	+++	+	+
MHA30	0,38±0,01^{lm}	21,73±1,19^{j}	281,29±6,71^{lm}	++	+++	-	+
NHC12	0,75±0,01^{g}	8,01±0,91^{r}	572,05±5,56^{d}	+	+	-	+
NHA23	0,41±0,01^{l}	19,67±0,87^{k}	285,00±1,73^{lm}	-	-	-	+
SHB93	0,78±0,01^{g}	7,21±0,53^{r}	570,63±2,66^{d}	+++	++	-	+
MHB51	1,24±0,03^{d}	11,35±0,53^{p}	679,86±8,37^{b}	-	+	-	+
NHA20A	0,02±0,00^{s}	20,75±0,07^{jk}	660,62±8,18^{bc}	+++	+	-	+
NHA68	0,16±0,00^{q}	19,98±0,91^{k}	804,67±4,04^{a}	-	++	-	+
NHA78	0,01±0,00^{s}	17,42±0,90^{m}	483,05±5,27^{f}	++	++	-	+
NHA66	2,19±0,01^{c}	14,80±0,25^{no}	364,47±4,08^{i}	++	++	-	+
NHB9	2,43±0,03^{b}	26,84±0,21^{g}	224,71±3,98^{n}	++	-	-	+
NMA27	0,11±0,00^{r}	23,57±0,27^{i}	320,95±4,65^{jk}	+++	+++	-	+
NMA14	0,05±0,00^{s}	20,59±0,41^{jk}	522,04±18,15^{e}	++	-	-	+
NHB19	0,17±0,01^{q}	10,10±0,76^{q}	490,38±10,00^{f}	++	++	-	+

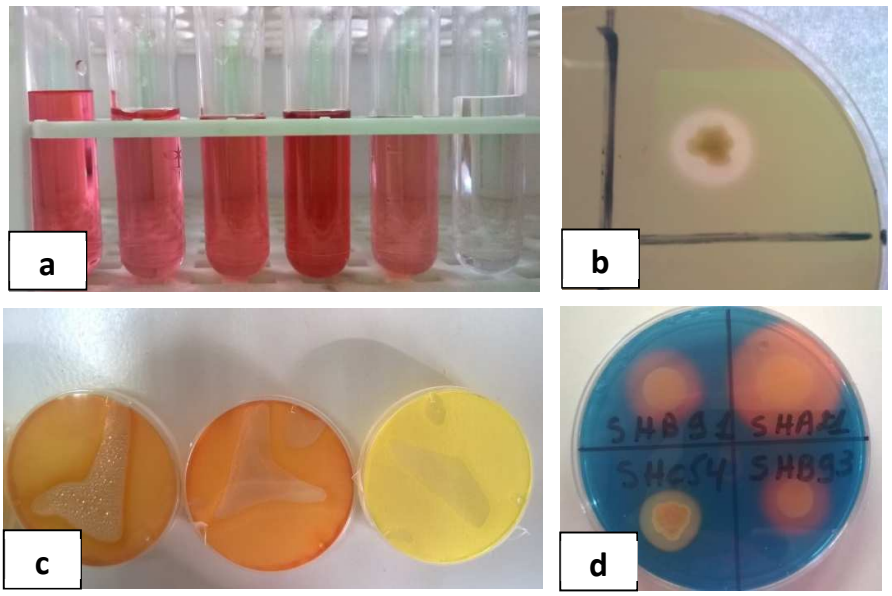

Figure1. PGP activities of the isolates, (a): IAA Production (b): P solubilisation (c): HCN production, (d): Siderophore production

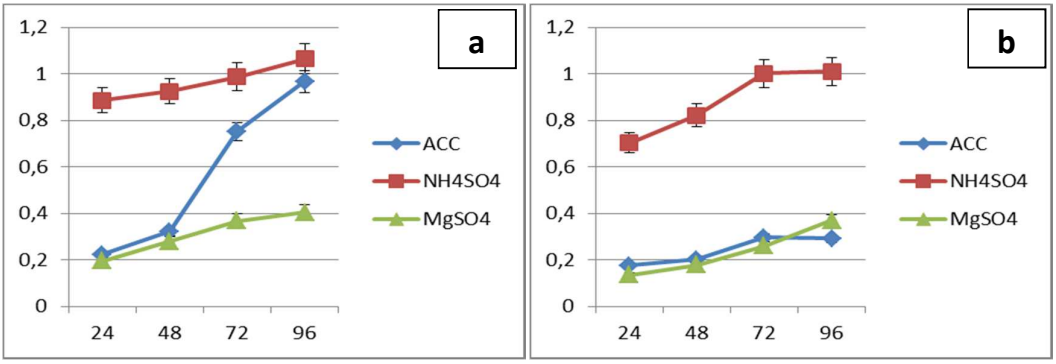

Figure 2. ACC metabolisme by the isolates, (a): NMA14 (positive), (b): SMA4 (negative)

PGPR traits of the isolated strains

The 35 isolates were tested for their ability to produce IAA, ammonia, siderophore, HCN, to solubilize phosphate or to use ACC as sol nitrogen source.

The 35 isolates were able to produce IAA (Figure 1a). Using the method of [17] with a medium supplemented with 200 mg/l of Tryptophan, the detected quantities of IAA produced by the isolated strains ranged from 5,05±0,83 µg/ml to 74,81±0,94µg/ml by the isolate NHA13 and NHA79 respectively. The majority of the values were over 20µg/ml and only 9 were under 10µg/ml (Table 2).

All the isolates were also able to solubilize phosphate by producing clear zones around the bacterial colonies on Pikovskaya medium (Figure 1b). In liquid broth media, the highest quantity of solubilized phosphate was detected for the isolate NHA68 with 804,67±4,04 µg/ml and the lowest quantity by isolate BHA0 with 47,67±5,51µg/ml. It should be noted that almost all the isolates showed high values for phosphate solubilization around 300 or 400µg/ml.

Table 3. Biochemical description and genotypic identification of isolates

Isolate	Gram reaction	Shape	Ox	cata	*Genius(16 rRNA sequence)*
NHA13	+ve	Rod	-ve	+ve	*Bacillus megaterium*
MHA24	+ve	Rod	-ve	+ve	*Bacillus megaterium*
BHA10	+ve	Rod	-ve	+ve	*Bacillus megaterium*
BHA0	+ve	Rod	-ve	+ve	*Bacillus megaterium*
NHA19	+ve	Rod	-ve	+ve	*Bacillus subtilis*
NHA79	-ve	Rod	-ve	+ve	*Enterobacter aerogenes*
NMB8	-ve	Rod	-ve	+ve	*Enterobacter aerogenes*
NHA67	-ve	Rod	-ve	+ve	*Enterobacter aerogenes*
BHA62	-ve	Rod	-ve	+ve	*Enterobacter aerogenes*
SHB91	-ve	Rod	-ve	+ve	*Klebsiella oxytoca*
SHB54	-ve	Rod	-ve	+ve	*Klebsiella oxytoca*
MHB62	-ve	Rod	-ve	+ve	*Klebsiella oxytoca*
MHC9	-ve	Rod	-ve	+ve	*Klebsiella oxytoca*
MHC54	-ve	Rod	-ve	+ve	*Klebsiella oxytoca*
MHB52	-ve	Rod	-ve	+ve	*Klebsiella oxytoca*
SHB57	-ve	Rod	-ve	+ve	*Klebsiella oxytoca*
NHA21	-ve	Rod	-ve	+ve	*Serratia odorifera*
SHA21	-ve	Rod	-ve	+ve	*Pseudomonas fluorescens*
NHA15	-ve	Rod	-ve	+ve	*Raoultella planticola*
MHA59	-ve	Rod	-ve	+ve	*Raoultella planticola*
MHB56	-ve	Rod	-ve	+ve	*Raoultella planticola*
SMA4	-ve	Rod	-ve	+ve	*Raoultella sp*
MHA30	-ve	Rod	-ve	+ve	*Raoultella sp*
NHC12	-ve	Rod	-ve	+ve	*Serratia odorifera*
NHA23	-ve	Rod	-ve	+ve	*Serratia odorifera*
SHB93	-ve	Rod	-ve	+ve	*Serratia odorifera*
MHB51	-ve	Rod	-ve	+ve	*Serratia odorifera*
NHA20A	-ve	Rod	+ve	+ve	*Sténotrophomonas maltophilia*
NHA68	-ve	Rod	+ve	+ve	*Sténotrophomonas maltophilia*
NHA78	-ve	Rod	+ve	+ve	*Sténotrophomonas maltophilia*
NHA66	-ve	Rod	+ve	+ve	*Sténotrophomonas maltophilia*
NHB9	-ve	Rod	+ve	+ve	*Sténotrophomonas maltophilia*
NMA27	-ve	Rod	+ve	+ve	*Sténotrophomonas maltophilia*
NMA14	-ve	Rod	+ve	+ve	*Sténotrophomonas maltophilia*
NHB19	-ve	Rod	-ve	+ve	*Enterobacter sp*

+ve: positive , -ve: negative, Ox: oxidase test , Cata: catalase test

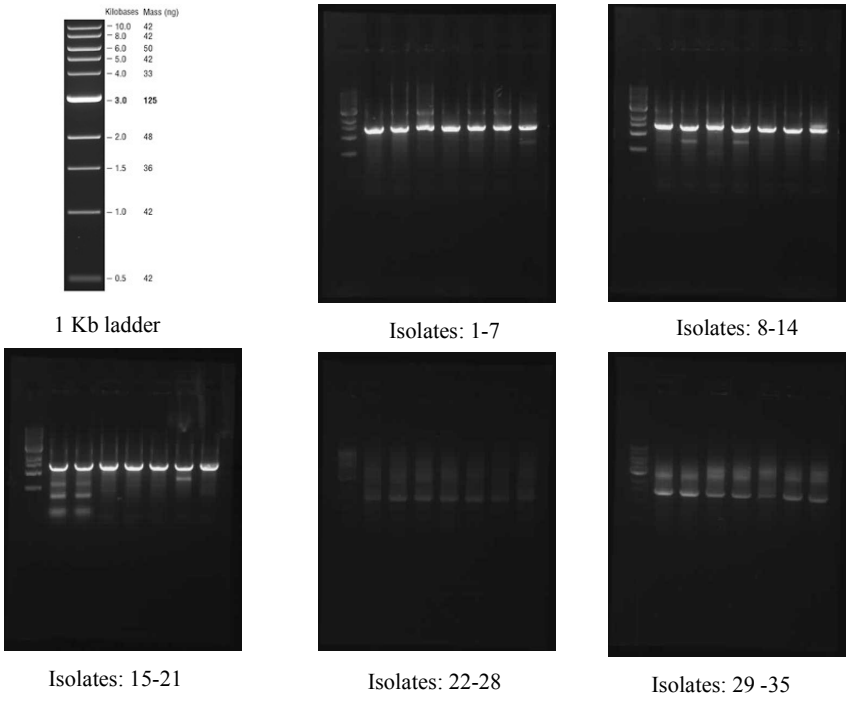

Figure 3. Amplification of 16S RNA gene. 1KB DNA Ladder

The five isolates MHC9, NHB9, SHB57, NHA23 and NMA14 were not able to produce siderophore. For the others, the diameters of yellow–orange halos around the bacteria colonies were not similar. The majority of the tested isolates showed a large halo but few showed a small one (Figure 1d and Table 2).

All the isolates were tested for ACC de-aminase activity by using ACC as the sole nitrogen source. Among 35 isolates, 19 grew well on DF medium with either ACC or ammonium sulphate serving as the sole nitrogen source. DF salt minimal medium without nitrogen source was used as a control (Figure 2).

Only two isolates (SH21 and SMA4) could produce HCN, however all strains were positive for ammonia production.

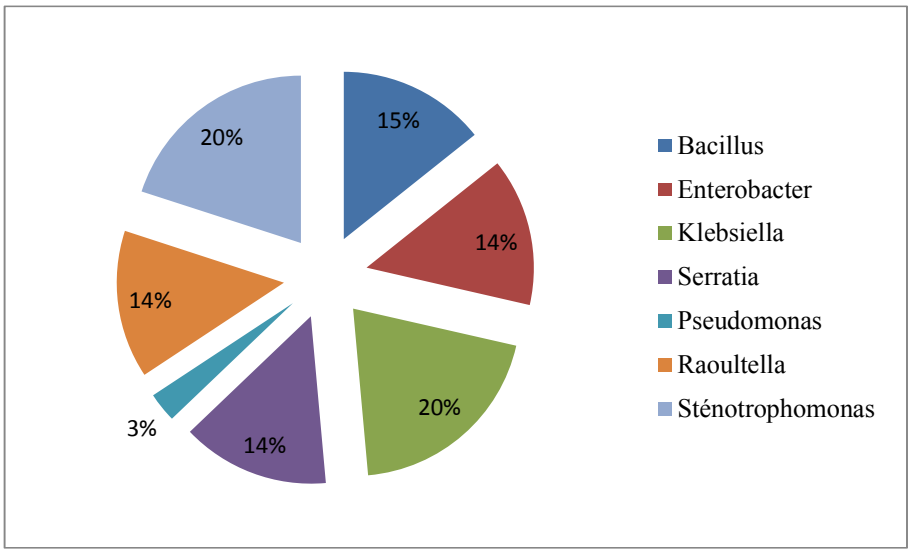

Figure 4. Diversity of isolates identified by 16S rRNA gene

Molecular identification

In order to better characterize the isolated rhizobacteria, their 16S rRNA gene sequence was determined. The 16S rRNA sequence was amplified and complete (1200 bp) or partial (900bp) sequences were obtained (Figure

3) and BLASTN-aligned with the sequences of different bacterial genera from the GenBank database (Table 3). This analysis of the sequences of 16S rDNA classified the isolates to the following seven bacterial genera: *Bacillus* (15%) *Stenotrophomonas* (20%), *Enterobacter* (14%), *Klebsiella* (20%), *Serratia* (14%), *Pseudomonas* (3%), *Raoultella* (14%), most of them (30) belonging to the division gamma-proteobacteria (Figure 4). The phylogenetic tree reflecting the relationship among the isolates is presented in Figure 5.

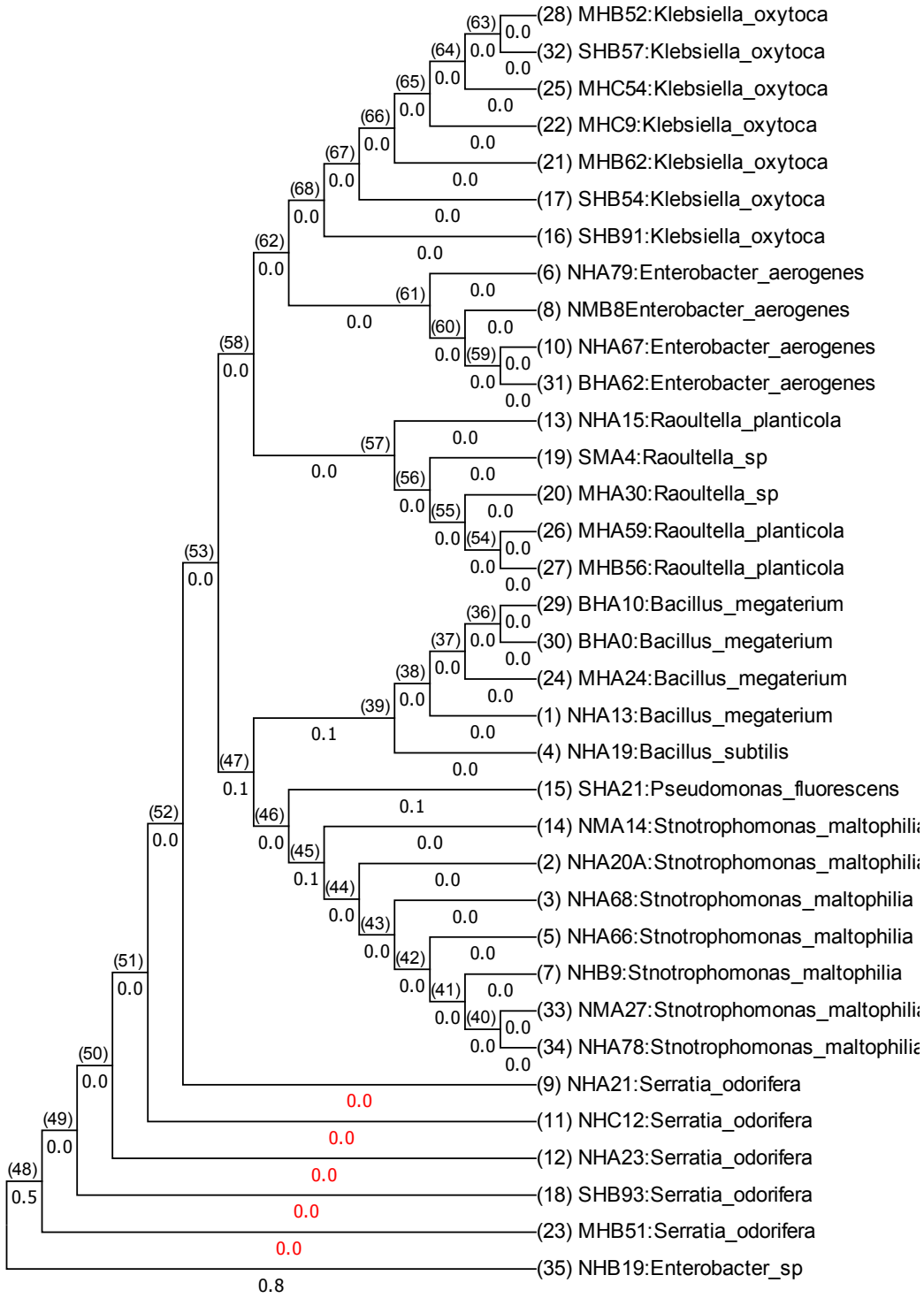

Figure 5. Phylogenetic relationships of isolates based on 16S rDNA sequence and related sequences. Neighbor-joining (NJ) tree was generated using the MEGA 6.0 software (www.megasoftware.net/). The numbers at the nodes indicate the levels of bootstrap support.

DISCUSSION

Rhizobacteria that have a combination of plant growth promoting traits, including phosphate solubilization, indole acetic acid and siderophore productions as well as nitrogen fixation capacity, have a potential to be used as PGP inoculants to increase crop yield production [27]. Many researchers all over the world have studied PGPR to understand how to use them to promote plant growth, to protect plants from pathogens and to benefit to agriculture. Various genera such as, *Azospirillum, Bacillus, Enterobacter, Microbacterium, Pseudomonas, Pantoea, Arthrobacter, Acinetobacter, Sphingobacterium, Rhodococcus and Xanthomonas* have been found in the rhizosphere of different plants [28, 29].

Isolating free-living diazotrophic bacteria from saline soil and screening all the isolates on the basis of their PGP activities were the focus of this work. Seven different genera of rhizobacteria (*Bacillus*, *Stenotrophomonas*, *Enterobacter*, *Klebsiella*, *Serratia*, *Pseudomonas, Raoultella*) were found in the rhizospheric soil of wheat plants in mina perimeter (west Algeria). *Bacillus* and *Stenotrophomonas* were previously described from wheat rhizosphere [30], Isolation of *Enterobacter, Azospirillum and Bacillus* from the rhizosphere of wheat has been reported from the same cropping fields in Pakistan [31]. *Serratia* and *Raoultella* with other genera were also found in the rhizosphere of Wheat in Rio Grande do Sul, Brazil [32].

Various genera including *Bacillus, Stenotrophomonas*, *Enterobacter*, *Klebsiella* and *Pseudomonas* were isolated from rhizosphere of Wheat plants in different parts of the NHZ of India [33].

The ability to reduce acetylene is an indirect measure of N_2-fixation. It is specific for monitoring functional Nitrogenase activity and is indicative of N_2-fixing potential [34]. Selective media and ARA test were used for the isolation and characterization of the different isolates described in this study. Previous investigations proved these method to be fast and simple [35] and effectively resulted in our work in the isolation of diazotrophs from several bacterial genus.

It is assumed that 80% of rhizospheric bacteria may produce plant growth promoting substances [36]. Saline soil is also considered as a rich source of bacteria that can produce IAA as 75% of the bacteria isolated from soil have the ability to produce IAA [37]. In our study, 68,82 % of the 296 isolated strains were IAA producers and from the 35 strains described in more details here the IAA production ranged from 5,05±0,83µg/ml to 74,81±0,94µg/ml. Such differences in the ability of IAA production may be attributed to the inherent properties of the individual bacteria [38]. This IAA production by PGPR is considered as very important for plant growth improvement.

Phosphate solubilization is an additional essential activity of plant growth promotion because bacteria solubilizing phosphate are making phosphate available for plants [39]. Amongst the 296 strains isolated from the wheat rhizosphere in our study 51% were able to solubilize Phosphate at different levels. The amount of phosphate solubilized by 35 bacteria studied here ranged from 47 to 804 µg/ml. In order to make the Phosphate available for plant, it must be hydrolysed into inorganic Phosphate by phosphatase. The major source of phosphatase in soil is considered as being from microorganism [40], in addition many studies support the role of PSB in making Phosphate available to plants.

The production of siderophores is another important trait of PGPR. Siderophores are iron chelators and an important key in increasing plant growth either directly by supplying iron to the plant or indirectly by influencing the plant growth. In addition, by producing siderophores binding to the available form of iron in the rhizosphere, the PGPR makes the iron unavailable to the phyto-pathogens and are consequently protecting the plant [3]. From the 296 isolates 39,45% produced siderophores and almost all of the selected 35 isolates were found to be siderophores producers.

Rhizobacteria containing ACC-de-aminases have been reported to decrease the inhibitory effects of ethylene both under saline stress [41]. These ACC-de-aminases hydrolyses ACC, the precursor of ethylene in plants into α-ketobutyrate and ammonia instead of ethylene [42, 43]. In this work, the 35 selected isolates were screened for ACC de-aminase activity and we found that 19 of them (54%) showed various level of ACC de-aminase activity.

Conclusion

Our results showed that diazotrophic bacteria isolated from saline soil in west Algeria in the rhizosphere of wheat had a high potential to produce growth promoting traits. Among the 35 isolates, two isolates of *Stenotrophomonas*, *Bacillus* and *Enterobacter* showed a high ability to fix nitrogen. The PGPR potentials of the strains isolated in this work were variable with the isolate NHA79 identified as *Enterobacter aerogenes* showing one of the best combined characteristics. The results described here suggest that the bacteria described in this work are exhibiting a combination of PGP activities that can be used as biofertilizer and might improve plant (wheat) growth and yield.

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