

Diversity of plant growth promoting Rhizobacteria of *Rhus tripartitus* in arid soil of Algeria (Ahaggar) and their physiological properties under abiotic stresses

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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Abstract: Plant Growth Promoting Rhizobacteria (PGPR) associated with Ucria (*Rhus tripartitus*) represents a good alternative for including this crop in revegetation programs in arid area. In this study, 137 bacterial strains were isolated in Tryptic Soy Agar medium (TSA) from six samples of ucria's rhizospheric soil (Ahaggar, Algeria), based on colony characteristics, Gram © reaction, oxidase and catalase tests. To evaluate their PGP activities and their physiological characteristics under stress environment, ten tests were made. Sixty strains of 16 genera were selected for their PGPR abilities, which represent 43.79% to the total of rhizobacteria isolated. The maximum bacterial population were *Bacillus* (35%). 71.66% of isolates were able to solubilize the phosphate, 31.66% were able to produce Indole Acid Acetic (IAA), 58.33% were siderophore producers, 28,33% were able to produce Cyanhydric Acid (HCN) and 70% were able to grow without any source of nitrogen. Indeed, PGPR strains have shown tolerance and/or resistance to several experimental environmental conditions. As a conclusion, the PGPR strains of Ucria's rhizosphere were shown a good potential for biofertilization and biocontrol of crops, and their tolerance to abiotic stresses is an interesting step to support their utilities.

1. Introduction

Rhus tripartitus (called Ucria, African sumac and Tahounek in tamahaq) is an important medicinal plant belonging to the Anacardiaceae fam-

ily. This species often grows in areas of marginal agricultural capacity. It encountered in Algeria, in arid areas especially in the mountains of the Ahaggar where the indigenous people (Touaregs) use it to treat gastric disorders (Cheramat and Gharzouli, 2015). This plant is also found in the North-Eastern part of Saudi Arabia and in Tunisia where it spreads in the center to the far southern part of the country. It is used in the Arabian traditional medicine for centuries to treat cardiovascular and gastrointestinal disorders and inflammatory conditions (Chetoui *et al.*, 2013; Shahat *et al.*, 2016).

The study of rhizosphere bacteria from medicinal plants is very important, as they are well known to have impact on plant growth and also produce industrially important metabolites and improve quality of medicinal products (Bafana and Lohiya, 2013). Considerable numbers of studies were focused on the beneficial effects of bacterial species that colonize the rhizosphere of many plant species and proved their beneficial effects on plant growth, yield, and productivity as well as their role in the reduction of their susceptibility to diseases caused by phytopathogenic bacteria, fungi, viruses and nematodes and even against abiotic stresses. These bacteria have been called «Plant Growth Promoting Rhizobacteria» (Kloepper *et al.*, 2004; Orhan *et al.*, 2006; Miransari, 2014; Nadeem *et al.*, 2014; Gupta *et al.*, 2015). These PGPR's can enhance the plant growth by direct mechanisms such as the fixation of atmospheric nitrogen, the solubilization of minerals like phosphorus and iron, the production of siderophores and enzymes, the synthesis of phytohormones like the auxin, indole-3-acetic acid (IAA), cytokinins and gibberellins, their role in lowering of ethylene levels and the induction of systemic resistance. Indirect mechanisms are used by PGPR to benefit the plant growth by the induction of the disease resistance by producing antibiotics or hydrogen cyanide, competition for nutrients, extracellular enzymes production and others (Glick, 1995; Vessey, 2003; Adesemoye *et al.*, 2009; Saharan and Nehra, 2011; Saha *et al.*, 2016).

To the best of our knowledge, there are no studies conducted on the rhizospheric bacteria associated with *Rhus tripartitus*, a medicinal plant that grows in Ahaggar (Algeria). We hypothesize, that this plant harbors a diverse group of rhizospheric bacteria that can help Ucria to cope with harsh environmental conditions. So, the main objectives of this study were to characterize the isolated rhizobacteria associated with the rhizosphere of *Rhus tripartitus*, their pro-

preties as plant growth promoting bacteria and their capacities to tolerate abiotic stresses.

2. Materials and Methods

Sample collection, isolation and characterization of rhizobacteria

Six soil samples are taken from the rhizosphere of the wild ucria shrubs from Ilaman region in Tamanrasset, an arid area, which is located in the National Culturel Parc of Ahaggar in the south of Algeria (22° 49' 59" north, 5° 19' 59" east) during March, 2017. Each soil sample was collected at a depth of 15 cm, around the root and placed in a sterile container. The samples collection was transported to the laboratory in an ice box set at 4°C.

Tenfold serial dilution of the samples was made by mixing the soil with sterile water, and plating on a Tryptic Soy Agar medium (TSA). The plates were inverted and incubated, at 30°C for five days. The maximum of bacterial colonies present on plates were purified and characterized. The Gram reaction, oxidase reaction and catalase test were performed as per standard procedure.

In vitro screening and identification of Plant Growth Promoting Rhizobacteria

The collected rhizobacterial isolates associated with *Rhus tripartitus* were tested for their capacities to produce plant growth promoting effects. The PGPR were identified by using API galleries E20, NE20, CHB, Staph and NH (API, bioMerieux sa, Lyon, France). All the strains were preserved in nutrient broth added with 20% glycerol at -80°C.

Nitrogen fixation. The fixation of molecular nitrogen is tested on a free nitrogen medium. The bacterial isolates are inoculated on the plates and incubated at 25°C for 24-48 h. The growth on this medium after being transferred ten times in the same medium reflects the ability of bacteria to fix nitrogen (Haahtela *et al.*, 1983).

Production of HCN. The strains ability to produce Hydro Cyanic Acid (HCN) is carried out according to the method of Lorck (1948) on solid bennett agar amended with 4.4 g/l glycine is inoculated with a loop of the bacterial culture. 90 mm Whatman paper are dipped in sodium picrate solution (0.5% picric acid and 2% sodium carbonate) for one minute and then placed underneath the Petri plates lids. The plated were sealed with parafilm and incubated at 30°C for four days. The appearance of an orange to

red color indicates the production of HCN.

Solubilization of phosphates. Qualitative phosphate solubilization activity was tested on NBRIP (National Botanical Research Institutes Phosphate) medium by applying a spot of 20 µl of bacterial suspension on the surface of the agar and incubated at 30°C for 15 days (Nautiyal, 1999). A clear halo zone around the colony is an indication of phosphate solubilization. The calculation of the solubilization index (S.I.) is carried out according to the formula developed by Kumar and Narula (1999):

S.I. = Diameter of the halo around the spot/Diameter of the spot

Production of siderophores. The production of siderophores on solid medium is carried out qualitatively on Chrome Azurol S (CAS) medium as described by Schwyn and Neilands (1987). The CAS plates were prepared and divided into Sectors and inoculated with bacterial culture spots (10 µl of 10⁶ CFU/ml) and incubated at 25±2°C for 48-72 h. The development of an orange yellow halo around the colony was considered positive for the siderophore production. The change in color is due to the transfer of ferric ions from the CAS to the siderophores. The calculation of the ratio (halo diameter/diameter of the bacterial colony) makes it possible to compare production differences between bacterial strains.

Production of indole acetic acid (IAA). The production of IAA was determined according to the method of Holt *et al.* (1994). The principle is to inoculate the selected strains on the nutrient broth containing 0.1 g/l of L-tryptophan. The change of the solution color from yellow to pink or red when we add the reagent of Salkowski (50 ml, 35% perchloric acid; 1 ml 0.5 FeCl₃) is an indication of positive result.

Screening of PGPR isolates for stress tolerance

Influence of salinity. To evaluate the strains ability of these strains to grow in salinity levels, different concentrations of NaCl (1%, 5% and 8%). The salt were added to TSA medium in the liquid stage (infection at 40°C) and deposited on the magnetic stirrer to dilute it.

Influence of pH and temperature. In order to test the ability of the bacteria to grow in alkaline and/or acidic environment, two media were prepared with the addition to 250 ml of TSA of 3 g of solid NaOH 6.4 M and 1 ml of KCl 12 N to obtain a pH of 8.8 and 6 respectively.

For studying the effect of incubation temperature

on growth of the isolates, the bacterial cultures were grown on TSA medium and incubated at a variables temperatures (10, 20, 30, 40 and 50°C).

Tolerance of heavy metal. The sensitivity of the selected strains to Copper (Cu), Zinc (Zn), Bromine (Br), Cyanide (Cn), Fluorine (F) and Silicon (Si) were tested. For this test, six TSA media of 250 ml were prepared with respectively three grams of solid KBr, CaF₂, SiO₂ and one gram of K₃[Fe(Cn)₆], ZnSO₄·7H₂O and CuSO₄·5H₂O. Any growth on this media reflects the ability of strains to tolerate the heavy metal toxicity.

Antibiotics resistance. The selected strains were tested for their susceptibility to antibiotics on Muller-Hinton medium. It consists of bringing the germ into contact with disks of blotting paper impregnated with a given antibiotic at concentrations determined by the standardization of the antibiogram according to the Clinical and Laboratory Standards Institute (CLSI), to determine the sensibility of this germ to antibiotics which allows classifying it in the category: R (resistant), S (sensible) or I (intermediate). Ten different antibiotics were used: Fosfomycin (FOS) 50 µg, Rifampin (RA) 5 µg, Nalidixicid (NA) 30 µg, Spiramycin (SP) 100 µg, 30 µg, Novobiocin (NV) 30 µg, Teicoplanin (TEI) 30 µg, Kanamycin (K) 30 µg and Erythromycin (E) 15 µg (all from Sigma Chemical Co., St. Louis, Mo.).

3. Results

In this study, we focused on the diversity of bacterial community of ucria's rhizosphere and the evaluation of their plant growth promoting abilities, also under abiotic stresses.

Isolation and characterization of rhizobacteria

One hundred and thirty seven (137) culturable bacteria were isolated from the rhizosphere of six healthy ucria plants using TSA medium. The rhizobacteria isolates showed a diversity of phenotypic and cultural characteristics of their colonies. Infact, 54.19% of the isolated rhizobacteria were Gram negative. 100% of strains showed positive test to catalase and variability to oxidase reaction (67.88% negative).

Screening and identification of PGPR

Biochemical characterization of PGPR isolates. The isolates were grouped into 16 genera of *Bacillus*,

Pseudomonas, *Ewingella*, *Staphylococcus*, *Alcaligenes*, *Micrococcus*, *Kocuria*, *Chryseomonas*, *Chryseobacterium*, *Cedecea*, *Shigella*, *Yersinia*, *Providencia*, *Acinetobacter*, *Haemophilus* and

Aeromonas based on cultural, morphological and biochemical characteristics. The maximum and minimum populations were *Bacillus* (35%), and *Haemophilus* and *Aeromonas* with a percentage of 1.66% each (Table 1).

Table 1 - Functional diversity of PGPR strains isolated from ucria's rhizosphere

| Sample No | Division/strains | Functions |
|-----------------------------|---|--|
| 1 | Gamma Proteobacteria | |
| | <i>Cedecea lapagei</i> | Solubilization of phosphate, production of HCN and siderophores |
| | <i>Chryseomonas luteola</i> | Solubilization of phosphate, production of siderophores and nitrogen fixation |
| | <i>Chryseomonas luteola</i> | Solubilization of phosphate, production siderophores and nitrogen fixation |
| | Firmicutes | |
| | <i>Bacillus megaterium</i> | Solubilization of phosphate, production of siderophores and nitrogen fixation |
| | <i>Bacillus megaterium</i> | Solubilization of phosphate, production of AIA and siderophores and nitrogen fixation |
| | <i>Bacillus circulans</i> | Solubilization of phosphate, production of AIA and siderophores, |
| 2 | Gamma Proteobacteria | |
| | <i>Ewingella americana</i> | Solubilization of phosphate, production of AIA and siderophores |
| | <i>Chryseomonas luteola</i> | Solubilization of phosphate, production of AIA and siderophores and nitrogen fixation |
| | <i>Cedecea lapagei</i> | Solubilization of phosphate, production of siderophores and nitrogen fixation |
| | <i>Cedecea lapagei</i> | Solubilization of phosphate, production of AIA and siderophores and nitrogen fixation |
| | Firmicutes | |
| | <i>Bacillus licheniformis</i> | Solubilization of phosphate, production of AIA and siderophores |
| | <i>Bacillus subtilis</i> | Solubilization of phosphate, production of siderophores and nitrogen fixation |
| | <i>Bacillus licheniformis</i> | Solubilization of phosphate, production of AIA and siderophores and nitrogen fixation. |
| | <i>Bacillus licheniformis</i> | Production of AIA and siderophores |
| | Actinobacteria | |
| | <i>Kocuria varians</i> | Production of AIA |
| 3 | Gamma Proteobacteria | |
| | <i>Chryseomonas luteola</i> | Solubilization of phosphate, production of AIA and siderophores and nitrogen fixation |
| | <i>Providencia rattgeri</i> | Production of AIA |
| | <i>Ewingella americana</i> | Production of siderophores |
| | Firmicutes | |
| | <i>Bacillus subtilis</i> | Production of HCN and fixation of azote |
| | <i>Bacillus non reactiv</i> | Production of siderophores and fixation of azote |
| | <i>Bacillus licheniformis</i> | Production of HCN and fixation of azote |
| 4 | Gamma Proteobacteria | |
| | <i>Yersinia pestis</i> | Solubilization of phosphate, production of siderophores and nitrogen fixation |
| | <i>Haemophilus aphrophilus</i> | Solubilization of phosphate, production of AIA, and nitrogen fixation |
| | <i>Aeromonas salmonicida</i> | Solubilization of phosphate, production of HCN and siderophores |
| | <i>Pseudomonas aeruginosa</i> | Solubilization of phosphate, production of HCN and nitrogen fixation |
| | <i>Cedecea lapagei</i> | Solubilization of phosphate, production of siderophores and nitrogen fixation |
| | <i>Acinetobacter colcoaticus</i> | Solubilization of phosphate and production of AIA, |
| | <i>Acinetobacter baumannii</i> | Solubilization of phosphate, production of AIA and nitrogen fixation |
| | <i>Chryseomonas luteola</i> | Solubilization of phosphate, production of HCN and siderophores. |
| | <i>Acinetobacter baumannii</i> | Solubilization of phosphate and nitrogen fixation |
| | Firmicutes | |
| | <i>Staphylococcus lentus</i> | Solubilization of phosphate and production of AIA. |
| | <i>Staphylococcus lentus</i> | Solubilization of phosphate, production of siderophores and nitrogen fixation |
| | Actinobacteria | |
| | <i>Micrococcus ssp</i> | Solubilization of phosphate, production of siderophores and nitrogen fixation |
| Béta Proteobacteria | | |
| <i>Alcaligenes faecalis</i> | Production of AIA and nitrogen fixation | |
| 5 | Gamma Proteobacteria | |
| | <i>Chryseomonas luteola</i> | Production of siderophores and nitrogen fixation |
| | <i>Chryseomonas luteola</i> | Solubilization of phosphate, production of AIA and siderophores and nitrogen fixation |
| | <i>Chryseomonas luteola</i> | Solubilization of phosphate, production of HCN and AIA. |

To be continued

Table 1 - Functional diversity of PGPR strains isolated from ucria's rhizosphere

continued

| Sample No | Division/strains | Functions |
|-----------|---|---|
| 5 | Firmicutes | |
| | <i>Bacillus circulans</i> | Solubilization of phosphate, production of HCN and siderophores and nitrogen fixation |
| | <i>Bacillus circulans</i> | Solubilization of phosphate, production of siderophores and nitrogen fixation |
| | <i>Bacillus licheniformis</i> | Solubilization of phosphate, production of siderophores and nitrogen fixation |
| | <i>Bacillus subtilis</i> | Solubilization of phosphate, production of siderophores and nitrogen fixation |
| | <i>Bacillus licheniformis</i> | Solubilization of phosphate, production of HCN and siderophores and nitrogen fixation |
| | <i>Bacillus licheniformis</i> | Solubilization of phosphate and nitrogen fixation |
| | <i>Bacillus circulans</i> | Production of HCN |
| | Bacteroidetes | |
| | <i>Chryseobacterium meningosepticum</i> | Nitrogen fixation |
| | Actinobacteria | |
| | <i>Kocuria varians</i> | Nitrogen fixation |
| | <i>Micrococcus ssp</i> | Solubilization of phosphate, production of HCN and siderophores and nitrogen fixation |
| 6 | Gamma Proteobacteria | |
| | <i>Ewingella americana</i> | Production of AIA and nitrogen fixation |
| | <i>Shigella spp</i> | Solubilization of phosphate and nitrogen fixation |
| | <i>Pseudomonas aeruginosa</i> | Solubilization of phosphate, production of siderophores and nitrogen fixation |
| | <i>Ewingella americana</i> | Solubilization of phosphate, production of siderophores and HCN and nitrogen fixation |
| | <i>Ewingella americana</i> | Solubilization of phosphate and nitrogen fixation |
| | <i>Providencia rattgeri</i> | Production of AIA |
| | Firmicutes | |
| | <i>Bacillus subtilis</i> | Solubilization of phosphate and nitrogen fixation |
| | <i>Bacillus licheniformis</i> | Solubilization of phosphate, production of HCN and siderophores and nitrogen fixation |
| | <i>Bacillus subtilis</i> | Solubilization of phosphate, production of HCN and nitrogen fixation |
| | <i>Bacillus non reactiv</i> | Nitrogen fixation |
| | Actinobacteria | |
| | <i>Micrococcus ssp</i> | Production of HCN and siderophores and nitrogen fixation |
| | Béta Proteobacteria | |
| | <i>Alcaligenes faecalis</i> | Solubilization of phosphatase, production of HCN and siderophores |
| | <i>Alcaligenes faecalis</i> | Production of HCN |

The Rhizobacteria were identified through six samples and five Plant Growth Promoting treatments: solubilization of phosphate, production of Cyanhydric Acid, production of Indol-Acid-Acetic, fixation of nitrogen and production of siderophores.

Plant growth promoting traits. The isolated rhizobacteria were screened for various PGP features responsible for plant growth promotion. The PGPR isolates represent 43.79% of the total of the rhizospheric bacteria. They belong to four divisions (Gamma-Proteobacteria, Firmicutes, Actinobacteria and Béta-Proteobacteria). The strains mostly belonging to the Gamma-Proteobacteria, most of them affiliated to the Enterobacteriaceae. According to the figure 1, species of Gamma Proteobacteria division were predominant with 45% among the isolats of PGPR of Ucria behind firmicutes with 38.33%. Infact, the species *Cedecea lapagei*, *Chryseomonas luteola* and *Ewingella americana* were the most representative PGPR of Gammaproteobacteria division. The species *Chryseomonas luteola* found in five samples of six rhizospheric soils (Table 1). Different combinations of PGP effects have been found, 18.33% strains have been able to produce up to 4 PGP effects against 40% were able to produce 3 PGP effects, 25% showed 2 traits of PGP and 16.66% were able to produce only one PGP effect. In fact, the genera *Bacillus*, *Ewingella*, *Alcaligenes*,

Chryseomonas and *Cedecea* have showed positive screening for all the PGP traits, according to the Table 2.

Screening and assessment of phosphate solublizers and IAA producers. Among the total isolates screened for phosphate solubilization, 71.66% were able to solubilize inorganic phosphate (Fig. 2) and were identified as potential phosphate solubilizing

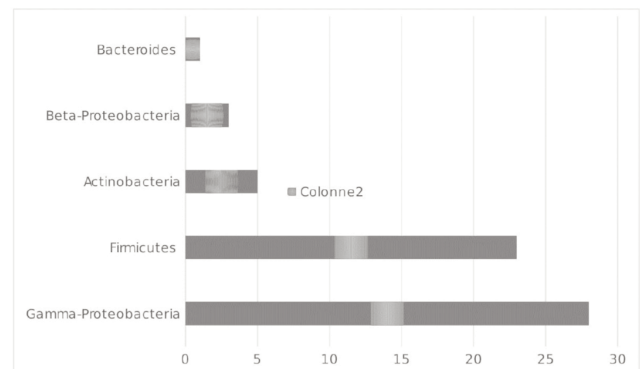


Fig. 1 - Classification of rhizospheric bacteria of Ucria on the PGP traits. The main bacteria divisions of plant growth promoting rhizobacteria from the rhizosphere of ucria.

Table 2 - Plant growth promotion activities of rhizobacteria genera isolated from ucria's rhizosphere

| Genera | N ₂ | Fe ²⁺ | HCN | AIA | PO ₂ |
|-------------------------|----------------|------------------|-----|-----|-----------------|
| <i>Bacillus</i> | + | + | + | + | + |
| <i>Pseudomonas</i> | + | + | + | - | + |
| <i>Ewingella</i> | + | + | + | + | + |
| <i>Staphylococcus</i> | + | + | - | + | + |
| <i>Alcaligenes</i> | + | + | + | + | + |
| <i>Micrococcus</i> | + | + | + | - | + |
| <i>Kocuria</i> | + | - | - | + | - |
| <i>Chryseomonas</i> | + | + | + | + | + |
| <i>Chryseobacterium</i> | + | - | - | - | - |
| <i>Cedecea</i> | + | + | + | + | + |
| <i>Shigella</i> | + | - | - | - | + |
| <i>Yersinia</i> | + | + | - | - | + |
| <i>Providencia</i> | - | - | - | + | - |
| <i>Acinetobacter</i> | + | - | - | + | + |
| <i>Haemophilus</i> | + | - | - | + | + |
| <i>Aeromonas</i> | - | + | + | - | + |

N² = nitrogen fixation, Fe²⁺= siderophore production, HCN production, AIA and PO= phosphate solubilisation.

bacteria that showed a clear halo zone around the colonies on NBRIP's agar plates amended with bromophenol blue. 20 isolates out of total rhizospheric flora had the capacity to produce were IAA in the presence of L-Tryptophane.

Molecular nitrogen fixation. The ability of the isolates to grow on N-free medium indicated positive results for nitrogen fixation. In fact 70% of the isolates were able to grow even being transferred ten times in this medium which indicated their capacity to fix molecular nitrogen (Fig. 2).

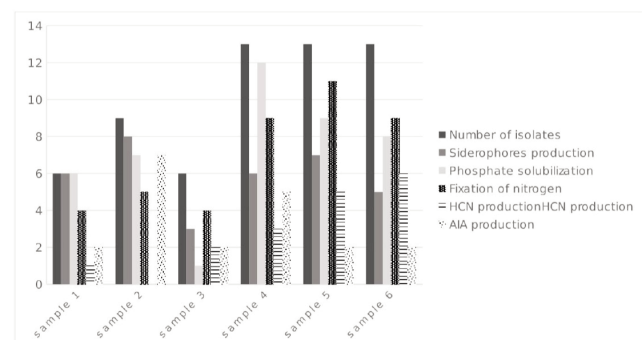


Fig. 2 - Siderophores production, phosphate solubilization, fixation of nitrogen, HCN and AIA productions of the Rhizobacteria isolates of ucria (Ahaggar).

Siderophore and HCN production. Siderophore production was registered at 58.33% of the strains based on the appearance of a halo zone of yellow orange color around the colony inoculated on CAS-agar plates. Seventeen isolates were positive for HCN production (Fig. 2).

Physiological properties of PGPR isolates under abiotic stresses. The isolated bacteria were tested for

their ability to tolerate abnormal growth conditions after incubation in a wide range of salt, pH and temperature stress condition, heavy metal toxicity and antibiotics.

Influence of salinity. According to the figure 3, the most percentages of strains which can grow in the 1% and 5% salinity are respectively 96.66% and 81.66%. In addition, only 16.66% of strains can tolerate % of salinity.

Influence of pH and temperature. The rhizobacterial isolates seems to tolerate the alkalinity better than the acidity with 66.66% and 46.66% respectively. However, these isolates could grow up to higher temperature of 50°C with 85%, which means that the temperature hadn't a remarkable effect on bacterial growth (Fig. 3).

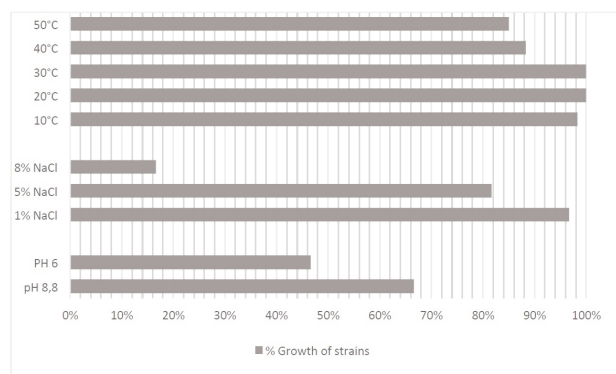


Fig. 3 - Estimation of tolerance of PGPR strains to alkalinity, acidity, salinity and temperature.

Tolerance to heavy metal. Depending on the metal tested and the species, the tolerance to heavy metals is different. In fact, we recorded a very good tolerance to silicon and bromine with respectively 80% and 78.33%. 55% of our strains have well tolerated fluoride followed by 30% for cyanide. The lowest percentages recorded were for copper and zinc with 13.33% and 3.33%, respectively. These results showed that heavy metals affected the bacterial growth (Fig. 4).

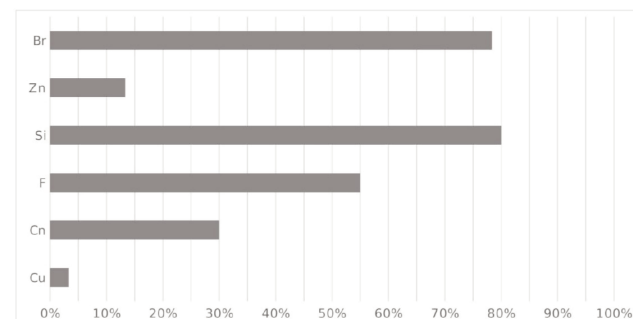


Fig. 4 - Estimation of tolerance of PGPR strains to heavy metals toxicity. Br= Bromine, Zn= Zinc, Si= Silicon, F= Fluorine, Cn= Cyanide, Cu= Copper.

Antibiotics resistance. The collection of PGPR isolates was tested for susceptibility to 10 antimicrobials. High frequency of resistance was observed for Métronidazole (MT) followed by Amoxyclav (AMC) with respectively 93.33% and 91.66% of strains. The minimum resistance was recorded for Fosfomycin (FOS) with 16.66% (Fig. 5).

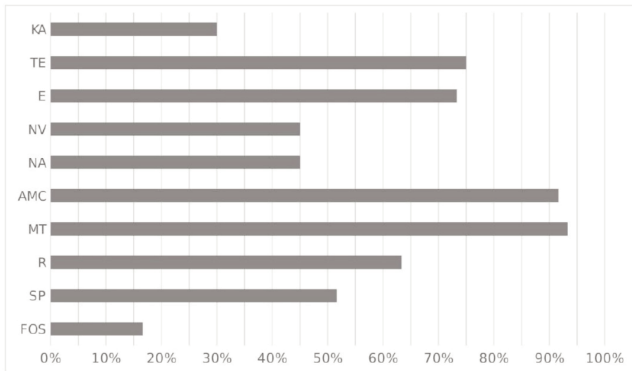


Fig. 5 - Estimation of resistance of PGPR strains to antibiotics.

4. Discussion and Conclusions

The diversity of native bacterial species in the southern Algerian soils remains largely unknown, especially for the rhizosphere of *Rhus tripartitus*. Most of the rhizobacteria isolated from ucria's rhizospheric soils have been dominated by Gram-negative (54.19%) and all were catalase positif. These results are in-line with studies realized on rhizosphere of *Brassica campestris* (Poonguzhali et al., 2006) and *Eragrostis tef Zucc. Trotter* (Woyessa and Assefa, 2011).

Plant growth promoting rhizobacteria (PGPR) represent a diverse range of soil bacteria that stimulate the growth of their host when grown in association. Such rhizosphere microbes benefit by utilization of metabolites secreted by plant roots as a nutrient for their growth and promote plant growth through more than one mechanism, including production of phytohormones and biocontrol of plant pathogens (Rana et al., 2011).

This present study showed a high diversity among the isolates of these species under different combination of PGP effects. The most often genera identified was *Bacillus* (35%) with a high diversity of species, such as *Bacillus megaterium*, *B. licheniformis*, *B. subtilis*, *B. circulans* and *B. non-reactiv*. PGPR of the genera *Bacillus* have been reported in many studies (Trivedi and Pandey 2008; Zou et al., 2010; Liang et al., 2011; Woyessa and Assefa, 2011;

Nadeem et al., 2012; Mishra et al., 2014; Susilowati et al., 2015). The second PGPR group found throughout this study was belonging to species *Chryseomonas luteola* (11.6%) and *Ewingella americana* (8.3%). The latter was able to increase the growth of piper and spinach (Hou and Oluranti, 2013). The third and last PGPR group (45.1%) belonged to the genera *Cedecea*, *Providencia*, *Yersinia*, *Heomophilus*, *Aeromonas*, *Acinetobacter*, *Micrococcus*, *Alcaligenes*, *chryseobacterium*, *Shigella*, *Staphylococcus* and *Pseudomonas*. Earlier studies showed that *Pseudomonas* PGPR is a producer of HCN (Castric, 1975), improving the availability of necessary nutrient (Islam et al., 2014) and an agent of biocontrol (Weller and Thomashow, 1993). The genera *Acinetobacter* promotes production of wheat, pea, chickpea, maize and barley through nitrogen fixation, siderophore production and mineral solubilization (Gulati et al., 2009; Sachdev et al., 2010). In contrast, *Cedecea* have never shown in the litterature as PGPR potential and molecular analysis is necessary to confirm the identification.

The industrialization of chemical fertilizers such phosphate and nitrogen has increased in the agricultural sector. These minerals are considered important limiting factors for many crops (Ahmad et al., 2008). However, the aim of this study is also to evaluate the ability of rhizobacteria to promote the biodisponibility of N and P, in order to reduce of industrial fertilizers. In fact, most of ucria's rhizobacteria could fix atmospheric nitrogen (70%) and 71.66% were able to solubilize inorganic phosphate in NBRIP's medium. Similary, many studies shown that phosphorus-solubilizing microorganisms are ubiquitous in soils (Chandra et al., 2007; Tsavkelova et al., 2007; Banerjee et al., 2010). PGPR can produce auxin-like compounds that increase the development of root system thus improving nutrient uptake by plants (Voisard et al., 1989); However, out of total isolated rhizobacteria, 31.66% exhibited as producers of IAA in medium supplemented with l-tryptophan. The number of PGPR producers of HCN represent 28.33% of total isolates strains. These PGP traits have the capacity to enhance indirectly plant growth and protect them from phytopathogens (Lugtenberg and Dekker, 1999; Shahat et al., 2016). Rhizobacteria producing siderophores are of great importance for the plant because they make bioavailable iron. The siderophores decreases the metal bond and formation of free radicals in the roots zone, which prevented the degradation of IAA (Yang et al., 2009). Among the isolates of PGPR obtained in this study, 58.33%

are producers of siderophores. The production of siderophores in the rhizosphere increases the bacterial competition as well the root colonization (Abrol *et al.*, 1988).

The rhizosphere is characterized by large environmental fluctuations, which may promote high diversity in the rhizosphere microbial community by maintaining high niche diversity. Thus, microbial community diversity may be important especially in extreme condition, like high temperature, salinity or pH changes. Under stress conditions, bacterial rhizosphere may promote the plant growth (Cheikh and Jones, 1994). In the present research, these PGPR isolates were traied under several environmental conditions. They have showed a good potential of tolerance for the previuosly conditions, which is a value, added to their beneficial effect. Therefore, the aim of this work is not only to deal with the diversity of PGPR but also the selection of resistant strains at the most extreme conditions.

The ucra's rhizobacteria, were isolated from an arid area of Ahaggar (Algeria) and salinity is a natural feature of ecosystems in arid and semi-arid regions (Curl and Truelove, 1986). The 81.66% of PGPR studied exhibited as tolerant to the presence of 5% NaCl and 85% could growth until 50°C. The variation on temperature is an important factor that can affect the hormonal balance of the plant (Lovley, 1995). Then, certain beneficial microorganisms can influence plants response to abiotic stresses like drought and high temperature (Grover *et al.*, 2011). Therefore, the necessity of discovering species able to grow under salt stress conditions and in a high temperature, are important to include them in revegetation system in arid area. The pH is one the obvious influencing factors of microbial activity and populations in soil (Woyessa and Assefa, 2011). Most of these isolates (66.66%) can growth over alkaline pH and more than 46% of growth recorded in acidity condition, it suggests that there is a good potential to inoculate them over a range of wide pH. The growth of rhizobacteria at acidic pH values could be explained by their adaptation at arid soil. Effectively, low rainfall can probably cause an increase in acidity of soil. On the other hand, an acidic environment of roots due to CO₂ and organic acid can be included in soil acidity (Gururani *et al.*, 2012).

Moreover, the PGPR isolates of this work, demonstrated a good tolerance *in-vitro* for heavy metals toxicity, with an average of 43.33% for chemicals forms of six metals. The accumulation in soil of heavy metals can perturbate the growth and the diversity

of bacterial communities. Many studies are shown the potential of application of PGPR in resistance and uptake of heavy metal by certain plants (Lovley, 1995; Yang *et al.*, 2009; Gururani *et al.*, 2012). The ability to colonize roots and antibiotic resistance are other parameters needed to detect effective PGPR strains (Siddiqui, 2005). Effectively, the study of the sensitivity of rhizobacteria to antibiotics adds PGP potential. Indeed, our strains have showed some resistance to the majority of antibiotics tested which can be involve high microbial competition in the rhizosphere. This resistance increased the chances of survival and colonization of the rhizospheric soil.

The present study reflects the preliminary work done on the rhizosphere of *Rhus tripartitus* in arid soils of Algeria. Indeed, the selection of PGPR strains, which can effectively grow under abiotics stresses conditions, can be used as promising biofertilizers and biocontrolling of plants and useful in revegetation system of arid area. Before that, others investigations should be done like a bioassay *in vitro* and *in vivo* of these strains on crops according to the inoculation treatments. The study of mechanisms of toleration to toxic substances or hard environment is an interesting step to support their utilities.

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