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Isolation and characterization of glyphosate-degrading bacteria from different soils of Algeria

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Glyphosate (N-phosphonomethylglycine) is the most commonly used herbicide worldwide. Due to the concern regarding its toxicity for non-targeted species in soil, finding glyphosate-degrading microorganisms in soil is of interest. The success of this will depend on isolating bacteria with the ability to grow in presence of glyphosate. Five bacterial strains were isolated from different untreated soils of Algeria, the strains were able to grow in a medium containing glyphosate as sole carbon or phosphorus source by enrichment cultures of these soils. Based on 16S rRNA gene sequence analysis, MALDI-TOF MS and biochemical properties, the best strain amongst them (Arph1) was identified as *Pseudomonas putida*. This isolate showed the highest growth level in the presence of glyphosate as sole phosphorus source. Arph1 was therefore used for further studies for optimization of cultivation conditions for an efficient glyphosate use. The best result of growth was on 1 g/L of glyphosate in minimal medium supplemented with glutamate with initial pH 9.0 at 30°C at 150 rpm within 168 h. Microbial growth during the study was monitored by measuring the optical density at 620 nm. Arph1 was able to tolerate up to 9 g/L of glyphosate. These results show that the bacterial strain may possess potential to be used in bioremediation of glyphosate-contaminated environments.

Key words: Soil pollution, glyphosate-degrading bacteria, *Pseudomonas putida,* optimization, cultivation conditions.

INTRODUCTION

The application of xenobiotic compounds generates environmental concern by the potential of the unwanted side effects, as large amounts of substances are released into the environment. The degradation of xenobiotic compounds is an important indicator for healthy ecosystems. Soil microorganisms can carry out pesticide degradation and can use the xenobiotic as a source of carbon, energy and other nutrients to promote microbial growth (Durkin, 2003). The herbicide glyphosate is often used to control weeds in grasslands. Despite its extensive use in Algeria, detailed informations on glyphosate degrading-microorganisms are lacking.

Glyphosate (N-phosphonomethylglycine) is the most commonly used herbicide worldwide (Franz et al., 1997); it is a broad-spectrum, post-emergence, non-selective herbicide, that inhibits the enzyme 5-enolpyruvylshikimic acid-3-phosphate synthase (EPSPS), blocking the synthesis of essential aromatic amino acids (Duke et al., 2003). The importance of glyphosate degrading-bacteria has been magnified by the biotechnology application. Effectively, the bacterial genes encoding for glyphosate-resistant EPSP synthase were cloned, endowed with

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chloroplast transit signals and used to transform plants (Della-Cioppa et al., 1987) to enable them to survive treatment following application of glyphosate. Use of this herbicide in glyphosate-resistant crops has given farmers cost-effective and broad-spectrum weed control options.

Several bacterial strains were isolated that were able to degrade glyphosate; most of these bacteria were isolated from sites already treated by the herbicide. However, there are few reports of the isolation of bacteria from untreated sites and no report of glyphosate degrading-bacteria isolated from Saharian soils. Previous reports were mainly focused on the screening of bacteria for their ability to degrade glyphosate. However, the comprehensive studies of the physiological regulation in bacterial cells are rather few (Shushkova et al., 2012). Thus, the optimization of cultivation conditions is important to appreciate this physiological regulation, and the identification of these conditions will make it possible to know which factors can be applied for bacteria in soil during bioremediation.

The aim of the present work was to isolate and characterize glyphosate-degrading bacteria by using enrichment cultures for three different untreated Algerian soils and the assessment of growth response of the isolates, as well as the optimization of some abiotic parameters for the cultivation of isolated strains, providing maximal effectiveness of the glyphosate degradation.

MATERIAL AND METHODS

Chemicals and media

The isopropylamine salt of glyphosate known as Roundup® (containing 450 g active ingredient/L of glyphosate, Monsanto) was purchased from a local store supplier of agricultural products in Constantine, Algeria.

For the isolation of bacteria using glyphosate as sole source of carbon and energy, mineral salt medium 1 (MSM1) was used. The composition of the medium in gram per liter of distilled water, pH (7.0 to 7.2) was: KH_2PO_4 (1.5), Na_2HPO_4 (0.6), NaCl (0.5), NH_4SO_4 (2), $MgSO_4$ $7H_2O$ (0.2), $CaCl_2$ (0.01) and $FeSO_4$ $7H_2O$ (0.001). Whereas, mineral salt medium 2 (MSM2) was used for the isolation of bacteria using glyphosate as sole phosphorus source, its composition in gram per liter of distilled water, pH (7.0 to 7.2) is: Tris buffer (12), glucose (10), NaCl (0.5), KCl (0.5) NH_4SO_4 (2), $MgSO_4$ $7H_2O$ (0.2) $CaCl_2$ (0.01) and $FeSO_4$ $7H_2O$ (0.001). Both media were supplemented with filter-sterilized (0.2 µm filter) glyphosate and were used to enrich and isolate glyphosate-degrading strains.

Experimental soil

Soil specimens were collected in April 2012 from three different untreated soils. The first sample was an agricultural soil, taken from the Institute of Field Crops in Constantine located between 7°35' longitude and 36°23' latitude in the center of eastern Algeria. The second sample was taken from the forest of Chaâberssas located in the University of Constantine, Algeria. The third sample was taken from a sandy field located in the region of Biskra located between 34°51'01" north latitude and 5°43'40" east longitude in the north-eastern of Algeria on the northern edge of the Sahara, Desert. Samples of about 1 kg were taken from the first 15 cm of depth, pooled and sieved. Samples were air dried and stored in sterile plastic bags at 4°C until use.

Enrichment and isolation of glyphosate-degrading strains

About 5.0 g of each soil were added to 95 mL of MSM1 or MSM2 medium in 250 mL flasks with the addition of glyphosate at a final concentration of 0.5 g/L and incubated in the dark at 30°C under shaking condition (150 rpm) for seven days. A 5 mL volume of these suspensions were then transferred to fresh MSM1 or MSM2 containing 1g/L glyphosate and incubated for seven days. Three additional successive transfers were made into media successively containing 3, 6 and 12 g/L of glyphosate. The appropriate dilutions of enriched samples were plated on plate count agar supplemented with 1 g/L glyphosate. The plates were incubated at 30°C for 24 h. Colonies were picked and purified. The strains Arph1, Arph2, Frglu, Frph and Bisglu were isolated.

Conventional identification

For morphological and physiological studies, Arph1, Arph2, Frglu, Frph and Bisglu isolates were grown in aerobically atmosphere at 30°C on Columbia Agar 5% sheep-blood media (Biomerieux, la Balm-les-grottes, France). Apart from morphology, mobility, catalase, oxidase and Gram reaction, physiological studies were performed by using API 20E.

MALDI-TOF MS identification

The MALDI-TOF mass spectrometry protein analysis was carried-out as previously described (Seng et al., 2009). Briefly, a pipette tip was used to pick one isolated bacterial colony from a culture Agar plate, and to spread it as a thin film on a MTP 384 MALDI-TOF target plate (Bruker Daltonics, Leipzig, Germany). Twelve (12) distinct deposits were done for each isolates from 12 different colonies. Each smear was overlaid with 2 µL of matrix solution (saturated solution of alpha-cyano-4-hydroxycinnamic acid) in 50% acetonitrile, and 2.5% tri-fluoracetic-acid, and allowed to dry for five minutes. Measurements were performed with a Microflex spectrometer (Bruker).

Spectra were recorded in the positive linear mode for the mass range of 2 to 20 kDa (parameter settings: ion source 1 (IS1), 20 kV; IS2, 18.5 kV; lens, 7 kV). A spectrum was obtained after 675 shots at a variable laser power. The time of acquisition was between 30 sand 1 minper spot. The 12 spectra of the different isolated strains were imported into the MALDI BioTyper software (version 2.0, Bruker) and analyzed by standard pattern matching (with default parameter settings) against the main spectra of 6.213 bacteria in the BioTyper database. For every spectrum, 100 peaks at most were taken into account and compared with spectra in the database.

16S rRNA gene amplification and sequencing

The 16S rRNA gene of the isolates was amplified using the primer pair fD1-P2 (Weisburg et al., 1991). PCR amplifications were carried-out in a 50 µL volume containing 5 µL template, 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM each dNTP, 0.2 µM each oligonucleotide primers and 0.5 units of *Taq* DNApolymerase (EuroblueTaq,

Eurobio, Les Ulis, France). The thermal cycle consisted of an initial 5 min denaturation at 95°C followed by 35 cycles of 30 s denaturation at 95°C, primer hybridization at 52°C for 30 s and elongation at 72°C for 1 min and a final 5-min extension step at 72°C. PCR reactions were examined by electrophoresing 5 µL of PCR product on a 1% agarose gel stained with ethidium bromide. The gel was visualized using Gel Doc 1000 (Bio-Rad, California, USA). Successful PCRs were transferred into PCR purification plate (Macherey Nagel HOERDT, France) filtrated with vacuum manifolds Millipore and agitated with a plate (Heidolph instrument Titramax 100). Purified PCR products were sequenced with the use of a BigDye® Terminator v1.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems), the Bvd1, 5X Sequencing Buffer and the primers 536F, 536R, 800F, 800R, 1050F and 1050R. Sequencing was then performed in ABI3700 automated capillary sequencer (Applied Biosystems, Foster City, California, United States).

The nucleotide sequences were edited using ChromasPro 1.34 software (Copyright (c) 2003-2006 by Technelysium Pty Ltd). Phylogenetic relationships of the genes were reconstructed using neighbor-joining implemented in MEGA 5 software (Tamura et al., 2011).

Glyphosate utilization patterns of the different isolates

Inoculums were prepared for each isolate by growing the strains in 50 mL of nutrient broth for three days at 30°C under shaking condition (150 rpm) till the growth reached late exponential phase. Cells were harvested by centrifugation at 4, 600 *g* for 5 min, washed with 0.9% sterile saline and were re-suspended to a 0.5 McFarland nephelometer standard (Optical density of 0.18 at 625 nm) and this was then used as the inoculum.

Growth experiments with glyphosate as the sole source of carbon or phosphorus were performed in 250 mL Erlenmeyer flasks containing 100 mL sterile MSM 1 or MSM 2 with 1 g/L of glyphosate. A 2 mL of each isolate was inoculated and triplicate cultures were incubated on a rotary shaker at 150 rpm for 168 h at 30°C. Non-inoculated media served as control. Samples (2 mL) were withdrawn periodically from the cultures to determine growth by measurement of the turbidity at 625 nm using a spectro-photometer.

Optimization of cultivation conditions

To optimize growth in glyphosate enriched media, some important abiotic factors were chosen. The important factors and their optimized ranges that were chosen in this experiment were nutriments (yeast extract, glutamate and glycerol), temperature (30, 37 and 40°C), medium pH (5.0, 6.0, 7.0, 8.0, 9.0 and 10.0) and initial concentration of glyphosate (1, 3, 5, 7, 9, 12 and 15 g/L). All experiments were performed in 250 mL flasks containing 100 mL of MSM2 supplemented with an appropriate amount of glyphosate, adjusted to an appropriate initial pH and inoculated with 2 mL of Arph1 strain. The flasks were then incubated at the appropriate temperature in the dark for seven days and stirred on a rotary shaker at 150 rpm. Controls without inoculation were kept in similar conditions. Bacterial growth was followed by taking a sample of 2 mL of cultures after every 24 h until 168 h of incubation and the optical density was measured at 625 nm.

In the first step, the flasks containing the minimum media were supplemented with 0.1%, w/v of various nutrients (yeast extract, glutamate and glycerol). In the second step, minimum medium supplemented with 0.1%, w/v of glutamate were incubated at different temperatures (30, 37 and 40°C). In the third step, minimum medium supplemented with 0.1%, w/v of glutamate were adjusted to different initial pH (5.0, 6.0, 7.0, 8.0, 9.0 and 10.0) and incubated at 30°C. Finally, minimum medium supplemented with 0.1%, (w/v) of glutamate with different concentrations of glyphosate (1, 3, 5, 7, 9, 12 and 15 g/L), adjusted to pH 9 were incubated at 30°C.

RESULTS

In the present study, five bacterial strains were isolated from different untreated soils of Algeria; these isolates have shown an ability to grow in a culture medium in the presence of the herbicide glyphosate as sole source of carbon or phosphorus. These isolates were named as follows: Arph1 and Arph2 isolated from the agricultural soil of Constantine in the medium containing glyphosate as sole phosphorus source; Frglu isolated from the forest soil of Constantine in the medium containing glyphosate as sole C source; Frph isolated from the forest soil of Constantine in the medium containing glyphosate as sole P source and Bisglu isolated from the Saharan soil of Biskra in the medium containing glyphosate as sole C source.

Strains identification

For the MALDI-TOF analysis, the obtained score of the isolate Arph1, was 2.5 close to the species *Pseudomonas putida.* The sequence of 1500 bp of the gene 16S rRNA was deposited in GenBank under Accession number KC582298. The phylogenetic tree showing the result of the 16S rRNA of Arph1 is represented in Figure 1. The sequence of the 16S rRNA gene of the isolate was 99.5% similar to the 16S rRNA gene of *P. putida* (GenBank Accession No. gb |EU439424.1|) and 99.4% similar to 16S rRNA gene of *P. putida* (GenBank Accession No. gb |EU439425.1|). The result of the analysis of 16S rRNA is consistent with that of MALDI-TOF, morphological and biochemical properties (Table 1). Therefore, the isolate was identified as *P. putida.*

MALDI-TOF scores obtained for the isolates Arph2 and Frglu were 2.4 and 2.3 near to *Enterobacter cloacae*, respectively. For the analysis of 16S rRNA gene, the isolate Arph2 showed a sequence similarity of 99.1% with *E. cloacae* (GenBank Accession No. gb |JF772064.1|) and 98.9% with *E. cloacae* (GenBank Accession No. gb |CP003737.1|) and *Pantoea agglomerans* (GenBank Accession No. gb |AY335552.1|) while the isolate Frglu showed a similarity of 99.3% with *E. cloacae* (GenBank Accession No. gb |JX307682.1|) and 98.8% with *E. cloacae* (GenBank Accession No. gb |EF059833.1|). The sequences of 1513 and 1288 bp of the gene 16S rRNA of the isolates Arph2 and Frglu were deposited in GenBank under the Accession numbers KC582299 and KC582300, respectively. Phylogenetic analysis of 16S rRNA gene

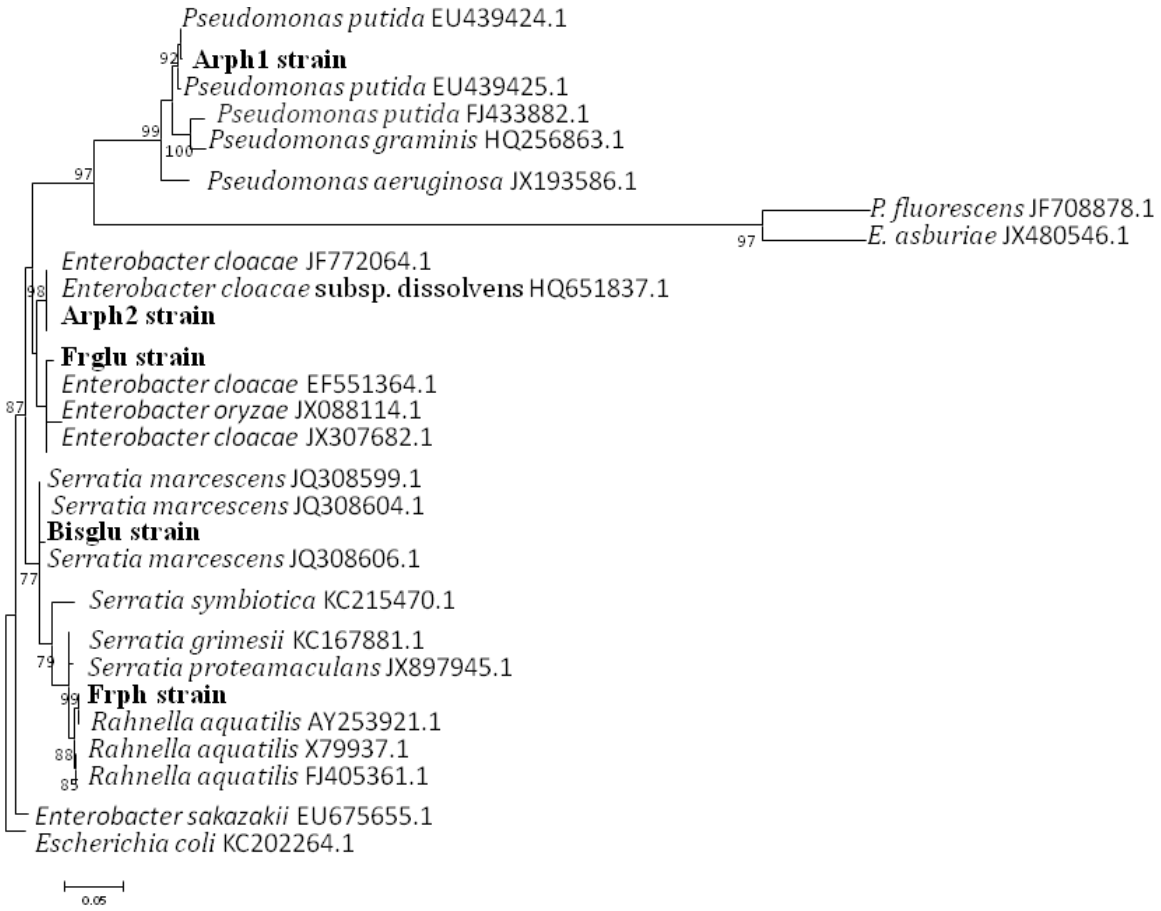

Figure 1. Neighbor joining tree based on 16S rRNA sequences for Enterobacteriacae**.** To examine the confidence of NJ tree, 1000 bootstrap replicates were used.

clustered the two isolates with *E. cloacae* species as shown in Figure 1. Based on the results of phylogenetic and phenotypic tests, the isolates Arph2 and Frglu were identified as *Enterobacter cloacae.*

The result of MALDI-TOF identified the isolate Arph3 as *Rahnella aquatilis* with a high score of 2.5. The result of the 16S rRNA gene analysis showed that the isolate Frph shares a similarity of 99.2% with *R. aquatilis* (GenBank Accession No. gb |FJ405361.1|) and 98.8% with *R. aquatilis* (GenBank Accession No. emb |X79937.1|). The sequence of 1337 bp of the gene 16S rRNA of the isolate was deposited in GenBank under Accession number KC582301. The result of the analysis of 16S rRNA gene of the Frph strain is consistent with that of MALDI-TOF, morphological and biochemical properties (Table 1). Therefore, the isolate was identified as *R. aquatilis.*

MALDI-TOF identified the isolate Bisglu as *Serratia marcescens* with a high score of 2.4. The result of the 16S rRNA gene analysis showed that the isolate Bisglu shares a similarity of 99.3% with *S. marcescens* (GenBank Accession No. gb |JQ308606.1|) and 98.9% with *S. marcescens* (GenBank Accession No. dbj |AB594756.1|). The sequence of 1233 bp of the gene 16S rRNA of the isolate was deposited in GenBank under Accession number KC582302. Based on the results of phylogenetic and phenotypic tests, the isolate Bisglu can be identified as *S. marcescens.*

Glyphosate utilization patterns of the different isolates

As shown in the Figure 2a, of the three isolates grown in the media containing the glyphosate as sole phosphorus source, and tested for the growth by measuring their turbidimetry at 625 nm, *P. pudida* showed the highest growth level (OD average = 0.129) suggesting extensive use of glyphosate. This was followed by *E. cloacae* (OD average = 0.100) and *R. aquatilis* (OD average = 0.084). However, for the isolates grown in the media containing the glyphosate as sole carbon source, the growth was very low as showed in the Figure 2b. The ODs averages

Table 1. Morphological and biochemical properties of the different isolates. (+), strains positive; (-), strains negative

Property	Arph1	Arph2	Frglu	Frph	Bisglu
Morphology	Rod-shaped	Rod-shaped	Rod-shaped	Rod-shaped	Rod-shaped
Motility	+	+	+	+	+
Biochemical tests					
Gram test	-	-	-	-	-
Oxidase/ catalase	+/+	-/+	-/+	-/+	-/+
β-galactosidase	-	+	+	+	+
Voges-Proskauer	-	+	+	+	+
Nitrate production	-	+	+	+	+
Lysine decarboxylase	-	+	+	-	+
Ornithine decarboxylase	-	+	+	-	+
H_2S production	-	-	-	-	-
Urease	-	-	-	-	-
Tryptophan deaminase	-	-	-	-	-
Indole production	-	-	-	-	-
Gelatinase	-	-	-	-	+
Arginine dihydrolase	+	-	-	-	-
Citrate	+	+	+	-	+
Sugar use					
Mannose	-	+	+	+	+
Glucose	-	+	+	+	+
Sorbitol	-	+	+	+	+
Rhamnose	-	+	+	+	-
Sucrose	-	+	+	+	+
Melibiose	-	+	+	+	+
Amygdalin	-	+	+	+	+
Arabinose	-	+	+	+	-
Inositol	-	-	-	-	+

were about 0.051 and 0.045 for *S. marscecens* and *E. cloacae,* respectively.

Effect of abiotic factors

Figure 3 shows the effect of certain nutrients (yeast extract, glycerol and glutamate) on bacterial growth. As seen in Figure 3 in the presence of glutamate, growth kinetic of *P. putida* strain shows a steady increase in growth after 24 h of incubation and reached a maximum of growth of 0.250 after 168 h of incubation, which represent an increase of over 15% compared to its growth in the medium without any nutriment. While in the presence of glycerol and yeast extract, the growth reached a maximum of 0.245 and 0.206 after 168 h of incubation, respectively. Therefore, the glutamate was selected as a carbon source for further studies.

Figure 4 shows the evolution of bacterial growth at different temperatures (30, 37 and 40°C). A significant increase in the growth of *P. putida* strain was noted at 30°C, where the growth of the strain reached its peak of 0.248 after 168 h of incubation. A less significant growth was obtained at 37°C, reached a maximum of 0.145 after 168 h of incubation. At 40°C, the strain showed a slower and weak growth and reached its maximum growth of 0.07 after 168 h of incubation. Therefore, the temperature 30°C was selected for further studies.

The effect of pH on the growth of *P. putida* is shown in Figure 5. In general, the growth of the strain is greater in alkaline pH ranging from 7 to 10 over 168 h. When the initial pH is lower than 7, the growth of the strain gradually decreased with the decrease of pH. At pH 9, the growth peaked significantly and reached a maximum of 0.261 within 168 h of incubation.

The growth kinetic at various initial concentrations of glyphosate is shown in Figure 6. Increase in microbial growth was observed till initial concentration of glyphosate was increased to 3 g/L. As the concentration of

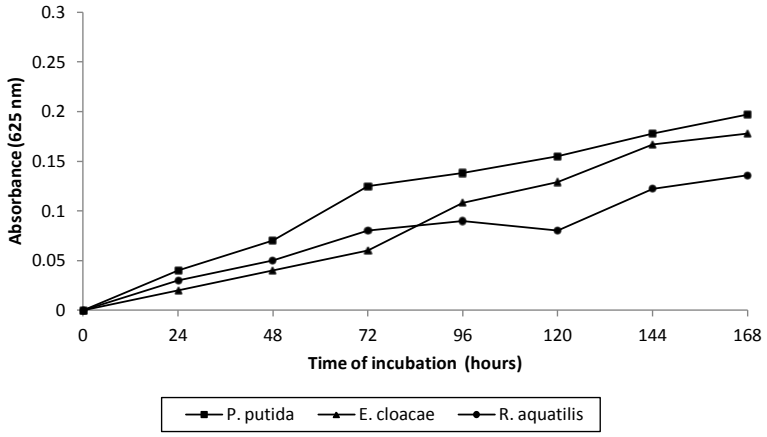

Figure 2a. Growth kinetics of *P.pudida*, *E. cloacae*, *R.aquatilis* strains in glyphosate as sole phosphorus source.

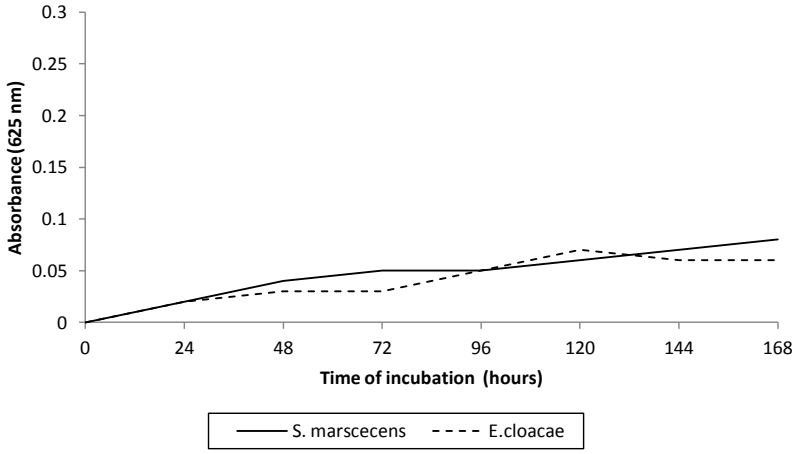

Figure 2b. Growth kinetics of *E. cloacae* and *S. marscecens* strains in glyphosate as sole carbon source.

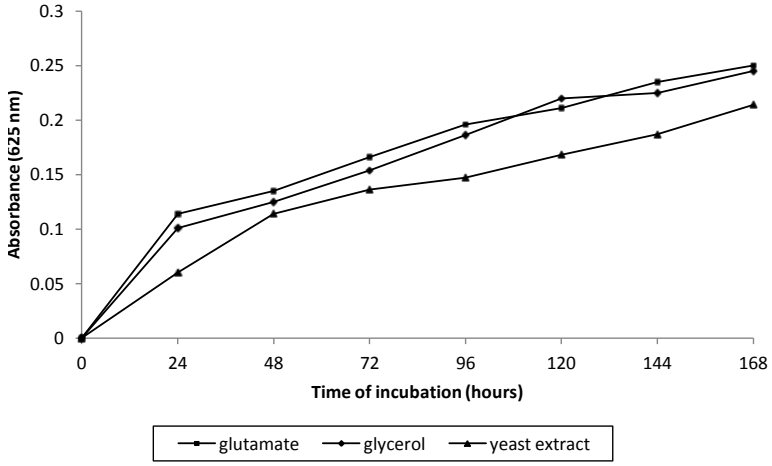

Figure 3. Growth kinetics of *P. putida* strain in glyphosate as sole phosphorus source with different nutrients.

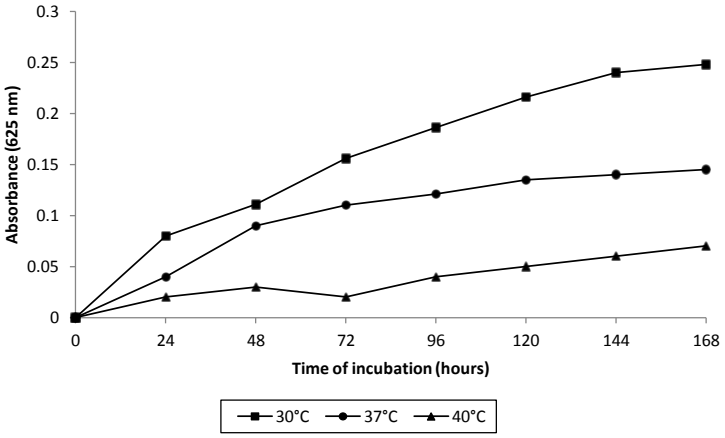

Figure 4. Growth kinetics of *P. putida* strain in glyphosate as sole phosphorus source supplemented with glutamate (0.1% w/v) in different temperatures.

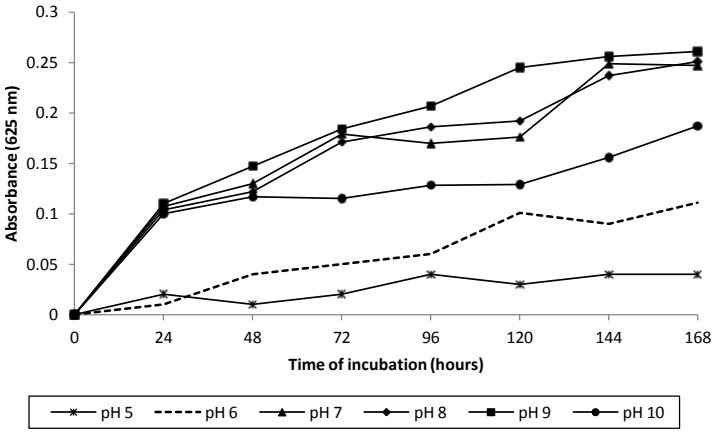

Figure 5. Growth kinetics of *P. putida* strain in glyphosate as sole phosphorus source supplemented with glutamate (0.1% w/v) with different pH at 30°C.

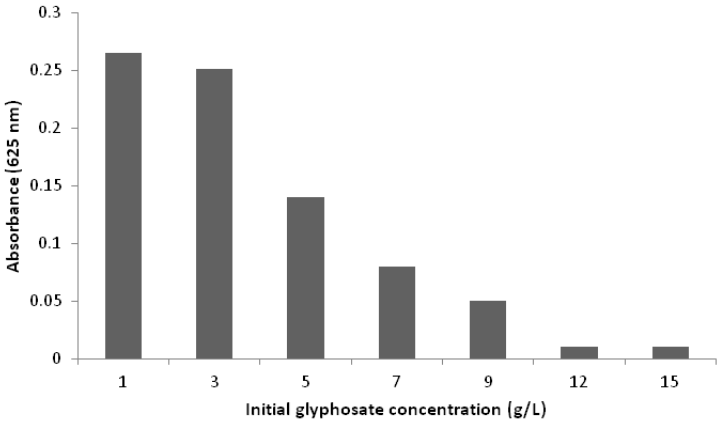

Figure 6. Effect of the initial concentration of glyphosate on the growth of *P. putida* strain.

glyphosate increased, there was a decrease in the growth of the isolate. The high concentrations of glyphosate severely inhibit bacterial growth.

The highest growth was observed at 1 g/L, which is the least tested concentration of glyphosate. After 24 h of incubation, the growth of *P. putida* in the medium containing 1 g/L of glyphosate increased significantly and reached a maximum of 0.265 after 186 h of incubation. However, No inhibition of growth was observed when initial gly-phosate concentration was increased further, indicating that the isolate can tolerate up to 9 g/L of glyphosate.

DISCUSSION

The limited number of strains isolated from the medium containing glyphosate as sole carbon or phosphorus source, is in agreement with the reports of Quinn et al. (1988), Santos and Flores (1995) and Kryzsko-Lupicka and Orlik (1997), which showed a significant reduction in microbial population when glyphosate was added to the medium culture. This result can be explained by the toxicity of artificial media due to the mode of action of glyphosate (the way of shikimic acid is ubiquitous in microorganisms (Glyphosate makes the organism unable to synthesize essential aromatic amino acids). In addition, Liu et al. (1991), Dick and Quinn (1995) report that when glyphosate is supplied as carbon source, microbial growth is rare, but growth stimulation is more apparent when applied in high concentrations. It was found that the commonly isolated glyphosate-degraders in the laboratory are the *Pseudomonas* spp. bacteria (Jacob et al., 1988; Dick and Quinn, 1995). Five *pseudomonas* species isolated that grew solely on glyphosate were identified (*P. maltophilia*, *P. putida*, *P. aeruginosa* and *Pseudomonas* sp.) that whose growth were not inhibited due to a glyphosate-resistant EPSPS (Schulz et al., 1985). As well, *Bacillus megaterium* (Quinn et al., 1989), *Alcaligenes sp.* (Tolbot et al., 1984) *Flavobacterium* sp. (Balthazor and Hallas, 1986), *Geobacillus caldoxylosilyticus* (Obojska et al., 2002), *Rhizobium* sp. and *Agrobacterium* sp. (Liu et al., 1991), *R. aquatilis* (Peng et al., 2012) and *Enterobacter cloacae* (Kryuchkova et al., 2013) have been reported as degrading glyphosate. However, there is no report of the isolation of *Serratia marcescens* as glyphosate degrading bacteria. Thus, this finding adds to the list of glyphosate-degrading bacteria a new degrading species that can be used in further studies.

The use of microorganisms for bioremediation requires an understanding of all physiological, microbiological, ecological, biochemical and molecular aspects involved in pollutant transformation (Iranzo et al., 2001). The effect of abiotic factors on bacterial growth was used to optimize the cultivation conditions that affect significantly the glyphosate degradation and the bacterial growth. *P. putida* was selected in this study because it showed the highest growth potential in comparison with the other species as showed in Figure 2a and b. The growth kinetics of *P. putida* was monitored over time at 620 nm using the MSM2 enriched with glyphosate as the sole source of phosphorus, varying abiotic conditions of the environment.

Growth rate was most important in the medium supplemented with glutamate; this results is in agreement with the report of Shushkova et al. (2012) who shows an effective glyphosate degradation in the presence of glutamate in the medium. Kumar and Philip (2006) reported that the addition of auxiliary carbon to the system having xenobiotic compounds increased the biodegradation potential of bacterial culture which was often because of increase in metabolic activity of the microbes involved. As reported by Mallick et al. (1999) and Guha et al. (1997), the co-metabolism appears to occur commonly in nature. Microbial activity increases with increasing temperature up to an optimum value. This result can be attributed to the fact that, at low temperature, the growth of the strain *P. putida* and the reaction catalyzed by the enzyme degrading glyphosate are increased. This indicates that the strain is psychrophilic nature (Patel et al., 2012). Moorman (1994) stated that within the range of temperature conditions normally encountered in cultivated soils, the rate of pesticide degradation generally increased with temperature. Walker et al. (1992) considered soil temperature to be the most important environmental factor influencing pesticide degradation rate in soils. It has been reported, that the incubation temperature does not only affect the pesticide degradation rate but also affect the growth of the strain.

Slightly alkaline pH is favorable for glyphosate degradation by the strain, probably due to the increased bioavailability and the decreased toxicity of glyphosate, and to the optimal metabolic activity of the bacterial cells. Singh et al. (2003a, b) report that in soils with higher pH a higher copy numbers of organo-phosphate degrading (*opd*) gene are found, suggesting that the activity of the enzymes degrading organo-phosphate compounds is more important at alkaline pH. Decrease in cell density at high concentrations of glyphosate can be attributed to the toxicity and the stress of glyphosate on strain. This can also be explained by the fact that at high concentrations, the appropriate catabolic enzymes may be repressed. Another plausible explanation is that the strain may need an acclimation period to induce the necessary degradative path (Tang and You, 2011). A similar resultwas found by Moneke et al. (2010) when testing different initial concentrations of glyphosate on *Acetobacter* sp. and and *P. fluorescens*. Tolerance to high pesticide concentrations is critical, since concentrations at contaminated sites may be several orders of magnitude higher than the

recommended usage doses for these products.

Conclusion

This study is the first report of isolation and characterization of a soil-borne bacterial strains (*P. putida, E. colacae*, *R. aquatilis* and *S. marcescens*) from an agricultural, Saharan and forest soil in Algeria that possess the capacity to use glyphosate. The capacity of these isolates to survive and grow in the presence of high concentrations of the herbicide, show that these strains may possess potential to be used in bioremediation of glyphosate-contaminated environments or moreover, can contribute on creating glyphosate-resistant crops.

In addition, this work adds to the list of glyphosate-degrading bacteria a new degrading species that is *S. marcescens*. This study provides also important information on optimization of critical parameters of cultivation conditions to enhance glyphosate degradation by *P. Putida* strain.

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