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# Phenotypic and Genotypic Characterization of Phosphate Solubilizing Bacteria Isolated from the Errachidia Province, Morocco

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***Authors' contributions***

*This work is a part of the PhD of the first author and was carried out in collaboration between all authors. Author BBM designed the study, authors BBM and IA performed the experiment and statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors JI and LN supervised the study and managed the literature searches, author EEF did the genotypic analysis. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** The aim of this study was to characterize phenotypically and genotypically bacterial isolated from the soils of the Errachidia province in order to select the ones having the potential to solubilize the inorganic phosphate.
**Study Design:** Rhizosphere soil samples for different legumes in sixteen sites from Errachidia province were collected for the assessment.
**Place and Duration of Study:** Department of Biology (Soil & Environment Microbiology Unit) Faculty of Sciences, Moulay Ismail University and Technical Support Unit for Scientific Research, CNRST in Rabat; between September 2012 and January 2013.

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**Methodology:** The samples were collected from 16 different sites belonging to Errachidia province in order to select bacterial strains able to solubilize inorganic phosphate. Morphological, cultural and phenotypic parameters of the isolated strains were evaluated. Phenotypic characteristics include the use of carbohydrates, tolerance to temperature, salt and pH. The genotypic diversity between the selected isolates was investigated throught 16S rDNA gene sequencing.
**Results:** 19,4% from the 62strains isolated were selected as PSBs; They showed phenotypic heterogeneity confirmed by the genetic one. Their effectiveness on the solid growth medium was greater which was confirmed by the significant index of solubilization of each strain. Although that on the broth medium the concentration of the solubilized phosphorus differs from one strain to another, a negative correlation moderately strong between the final pH of the growth medium and the concentration of the solubilized phosphorus was deduced. Also a lack of correlation between the index of solubilization and concentration was concluded (r = -0.08).
**Conclusion:** The importance of the phosphorus soluble forms for the agricultural production and taking in consideration the environmental concerns and sustainable developments compromise the use of cheapest and natural techniques to protect the environment.

*Keywords: Phosphorus; solubilization; inorganic phosphorus; phosphorus solubilizing bacteria.*

## 1. INTRODUCTION

Phosphorus (P) is one of the main macronutrients required for plant growth and both forms soluble and insoluble phosphate compounds are used as a source of fertilizer [1]. This macronutrient plays a key role in the plants energy transfer system. This element is generally abundant in soil, but because of its highly reactive nature it is immobilized and that make it unavailable to plants [2]; consequently the amount of phosphorus in its available form for the plants becomes a limiting factor [3]. Hence the use of the phosphate solubilizing bacteria can be a good solution to overcome this problem due to their effectiveness in the release of the inorganic as well as the organic forms of P from the total pools of the soil phosphate by solubilization and mineralization [4]. These forms of the phosphorus promote the plant growth [5]. Since the phosphate solubilizing microorganisms are common in soil [6], they were easily isolated in laboratory since the 1950s, using methods such as that described by Sperber [7]. So the purpose of this study was to selectively isolate phosphate solubilizing bacteria and to study their phenotypic as well as genotypic characteristics.

## 2. MATERIALS AND METHODS

### 2.1 Soil Sample Collection

Soil sampling Companies were conducted during the year 2012-2013. According to a completely random sampling, the whole province of Errachidia was covered; at each sampling site, 1000 grams of soil were taken at a depth ranging from 10 to 30 cm. All samples were preserved fresh for subsequent bacteria isolation and purification.

### 2.2 Isolation and Purification of Strains from Soil Samples

10 grams of each soil sample were suspended in 90 ml of sterile distilled water. 2 ml of the suspension of each suspension were flooded on the Yeast Mannitol Agar (YMA) plates and incubated at 28°C for seven days [8]. The purification of the bacterial cultures was ensured via successive subculturing on YMA medium until obtaining one type of single colonies.

### 2.3 Selection of Phosphate Solubilizing Bacteria

After purification, the strains were submitted to assess their ability to solubilize the phosphorus using NBRIP (National Botanical Research Institute's Phosphate) solid medium containing the tri-calcium phosphate (TCP) as the sole source of phosphate characterized by its insoluble form. After incubation at a temperature of 28°C for 10 days, the strains that showed a clear halo around the colony, indicating the phosphate solubilization, were selected for carrying out further tests.

### 2.4 Qualitative Assessment of Phosphate Solubilizing Bacteria

All strains showing a clear halo around the colonies on the NBRIP solid medium have been qualitatively estimated. Each phosphate solubilizing bacteria was suspended in the

NBRIP broth medium over-night. From each strain suspension, 10 µl were taken and deposited on the NBRIP solid medium; this step was repeated four times for each strain, and then the plates were incubated at temperature about 28°C. For each strain, the diameter of the halo and that of the colony were measured after 10 and 20 days of incubation. The solubilizing potential was assessed by calculating the index of solubilization (SI) which is the ratio of the diameter of the halo (colony + halo) to the diameter of the colony [9].

## 2.5 Quantification of the Phosphate Solubilized by the PSBs Selected

To estimate the amount of the phosphorus solubilized, seven strains showing high potential of solubilization were retained. Using a sterile loop, a colony was taken and suspended on the NBRIP broth medium overnight. From each tube, incubated at 28°C and put under stirring overnight, 1 ml of the bacterial suspension (at a concentration ranging from $10^8$ to $10^9$ CFU) [10] was transferred into a flask containing 150 ml of the NBRIP broth medium sterile. This medium contained tricalcium phosphate as the sole source of insoluble phosphate. The control flask contains only sterile broth medium. The flasks were incubated at a temperature of 28°C under agitation (180 rpm). Samples from the flasks were made for quantification and pH measurements (Metrohm 620 pH meter) at the launch of the test and every 24 hours for 7 days; at each sampling, 1 ml of the broth medium suspension was taken with three repetitions for each flask. Then samples were centrifuged at 10,000 rpm for 10 min. The supernatant from each strain was collected, sterilized by autoclaving at 121°C for 20 min and filtered with the Millipore Whatman paper (0.2 µm). Determination of phosphorus in each sample was done using the method of JE Harwood et al. [11].

## 2.6 Phenotypic Characteristics

### 2.6.1 Colony morphology

The morphology of isolates was evaluated on Yeast Mannitol Agar (YMA) plates which were incubated from 3 to 7 days at 28°C. The colonies were characterized on the basis of the size, color, shape, transparency, borders and elevation [12].

### 2.6.2 Congored test

The isolates were tested on YMA agar containing 1% of Congo red and incubated from 3 to 7 days at 28°C. The colonies were characterized based on the absorbance of the red coloration [13].

### 2.6.3 Bromothymol blue test

To test if a reacting strain is acidic or alkaline, all isolates were cultured on YMA plates containing 1.5% Bromothymol blue for 3 to 7 days. The change of coloration in the plates records the type of reaction [13].

### 2.6.4 Use of carbohydrate as carbon source

The use of different carbon sources was tested on YMA medium by replacing the mannitol with the carbohydrate to be tested. The carbohydrates tested were: Arabinose, glucose, maltose, mannose, starch and sucrose.

### 2.6.5 Carbohydrate metabolism

*2.6.5.1 Kligler-Hajna test medium*

The Kligler-Hajna Agar was used to differentiate the strains by identifying their ability to ferment glucose, with or without producing gas, to screen for lactose fermenting and/or hydrogen sulfide ($H_2S$) production.

*2.6.5.2 Simmons citrate test medium*

This medium was used to select the strains able to utilize Ammonium Dihydrogen Phosphate and Sodium Citrate as their sole sources of nitrogen and carbon.

*2.6.5.3 Mannitol motility test medium*

This semisolid medium already prepared by Bio-Rad at 4% of agar was used to detect the strain's motility.

### 2.6.6 Temperature tolerance

Temperature tolerance of the isolates was tested by incubating the inoculated YMA plates at 4, 28, 40 and 53°C.

### 2.6.7 pH tolerance test

The test of the capacity of the isolated strains to grow in acidic or alkaline media was determined

on YMA plates whose pH had been adjusted and buffered to 4.0, 5.0, 7.0, 9.0 and 11.0 [14].

#### 2.6.8 Salt tolerance test

All isolates were examined for their tolerance to salt on YMA supplemented with 0, 1, 2, 3, 4 and 5% of NaCl (w/v).

### 2.7 Genotypic Characteristics

The molecular study involved 12 selected bacteria and was carried out at functional genomics platform of the Technical Support Unit for Scientific Research, CNRST in Rabat-Morocco.

#### 2.7.1 Genomic DNA extraction of isolates

DNA extraction from bacterial strains on liquid culture using the kit "Gen Elute Bacterial Genomic DNA kit" from SIGMA, Aldrich according to the protocol provided.

#### 2.7.2 16S ribosomal DNA gene amplification

To amplify the 16S rDNA gene, two primers were used FD1 (5′ AGAGTTTGATCCTGGCTCAG 3′) and rp2 (5' ACGGCTACCTTGTTACGACTT 3') [15]. PCR amplification was carried out in 25 µl of the reaction volume containing template DNA (30 ng), Taq buffer (10 x), $MgCl_2$ (50 mM), dNTP mixture (10 mM), fd1 primer (100 µM), rp2 primer (100 µM), and 5 U/µl of Taq DNA polymerase. PCR amplification was performed with a «Verity» thermal cycler model from ABI ((Applied Bio systems, Foster City, USA). The PCR temperature profile used was 96°C for 4 min followed by 35 cycles consisting of 96°C for 10 s, 52°C for 40 s, 72°C for 2 min, with a final extension step at 72°C for 4 min. Reaction efficiency was estimated by horizontal agarose gel electrophoresis (1% w/v) using a molecular weight marker of 100 bp and photographed. The photos were displayed by the "G Box" photo documentation system.

#### 2.7.3 16S rRNA gene sequencing

Sequencing was performed on the 515 bp to 907 bp region of the 16S rRNA gene using the 3130XL Dye Terminator Cycle Sequencing (DTCS) Quick Start kit (Applied Biosystems) according to manufacturer instructions with 25–100fmol template DNA and 0.2 µM 515F and 907R primers (515F:GTGCCAGCMGCCGCGGTAA, 907R:CCGTCAATTCCTTTRAGTTT) [15]. For the purposes of this study, both strands of the 16S rDNA gene were sequenced for 12 samples, only the forward strand was sequenced for the remainder. The optimal thermocycling conditions for the cycle sequencing reaction were as follows: 25 cycles of 96°C for 1 min, 96°C for 10s, 50°C for 5s, and 60°C for 4 min, followed by a 4°C infinite hold.

The Sephadex G50 superfine (Sigma Aldrich) was used to remove unincorporated dye terminators from the cycle sequencing reaction, according to manufacturer's instructions with an additional 300 µl wash of the column with distilled $H_2O$ and centrifugation at 1500×g for 3 min prior applying the sample to the column.

DNA sequencing was performed on an ABI PRISM 3130XL Genetic Analyzer (Applied Biosystems) using the POP-7 polymer and ABI PRISM Genetic Analyzer Data Collection and ABI PRISM Genetic Analyzer Sequencing Analysis software Preliminary identification was performed by FASTA search of the Ezbiocloud database and a more precise identification was performed by phylogenetic analysis with type strains of the nearest neighborhoods.

## 3. RESULTS

### 3.1 Isolation of Strains from Soil Samples

In this study, 62 strains were isolated from soils sampled from different sites in the Errachidia province and then purified via successive subcultures.

### 3.2 Selection of Phosphate Solubilizing Bacteria "PSB"

The selection of the phosphate solubilizing bacteria was done via inoculating separately the 62 isolates into the NBRIP plates. Therefore, just 12 strains (22.6%) of the isolates were phosphate solubilizing bacteria. Based on their solubilization index, the selected strains were different in terms of their solubilizing power.

After 10 days of incubation, it's noticed a good phosphate solubilization by different strains tested; while noting that the strain 2 was the most efficient with 6.77 solubilization index, we also noticed that the lowest solubilization index (SI = 2.71) was that of the strain 9.

While after twenty days, we observed that the order obtained in the first step has changed; we noticed that the most efficient strain was the strain 12 with a solubilization index of 7.15; however the strain with the lowest solubilization index after the first ten days period became ranked fourth with a solubilization index of 3.88. Finally we noticed that the lowest index, after the second ten days period, was observed for the strain 7 with 2.65 as solubilization index (Fig. 1).

However, the lowest indexes obtained in the present study were important compared to the previous studies assessing other bacteria on solubilizing the inorganic phosphates [16].

### 3.3 Quantification of the Available Phosphate Solubilized by the PSB

From the results obtained with the qualitative assessment, seven strains presented the high solubilization indexes, namely the strains 12, 2, 6, 9, 5, 1 and the strain 10. The amount of phosphorus solubilized by each of these strains was evaluated, using the NBRIP broth medium over a period of 7days.

Nevertheless, the quantitative assessment revealed different results regarding those of the qualitative estimation.

The maximum of the phosphate solubilized quantity was reached at different periods of incubation for all the tested strains (Fig. 2). Hence the strains were classified into two classes, the first class of strains (strain 2, 5 and 10) that have reached their maximum rate of solubilization after 120 hours (5 days) of incubation and the second class of strains (strain 1, 6, 9 and 12) that have reached their maximum solubilization after 144h (6 days) of incubation (Fig. 3).

Once the maximum solubilization rate was reached, two types of curves of solubilization were classified; for the first type, the curves showed a decrease of the concentration of the solubilized phosphate in the medium immediately after obtaining the maximum solubilization (strains S1, S5, S6, S9 and S12), the second type was observed for the strains S2 and S10, characterized by the stability of the maximum value of the solubilized phosphorus.

Concomitantly with quantification, the pH of medium was followed up every 24 hours; an acidification was noticed given that the pH of the medium was initially adjusted to 7 (Fig. 4).

According to Fig. 5, a negative correlation moderately strong (r = -0.7) was deduced between the final pH and the maximum concentration of tricalcium phosphate solubilized in the medium. So, it's concluded that as the tricalcium phosphate solubilization was maximum, the acidification of the medium was important too.

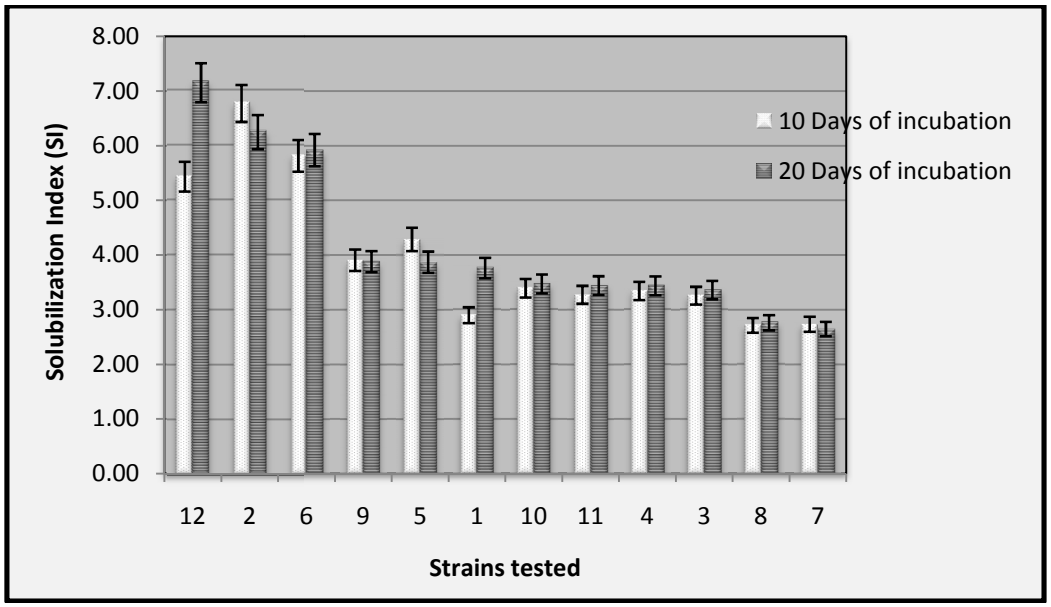


**Fig. 1. Variation of the solubilization index with the strain tested and the incubation period**

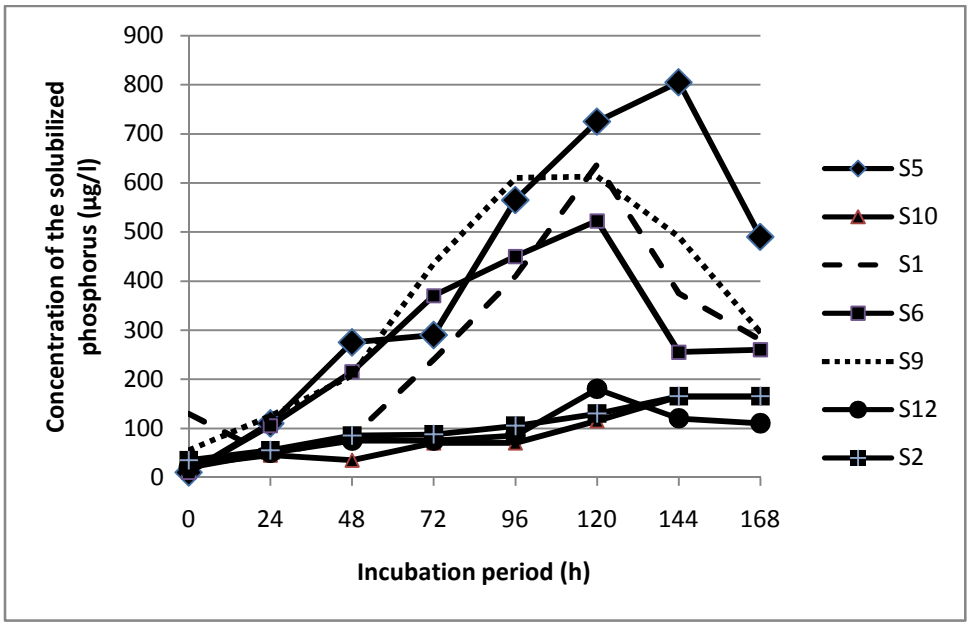


**Fig. 2. Variation of the amount of solubilized phosphorus with the incubation period and the strain tested (S=Strain)**

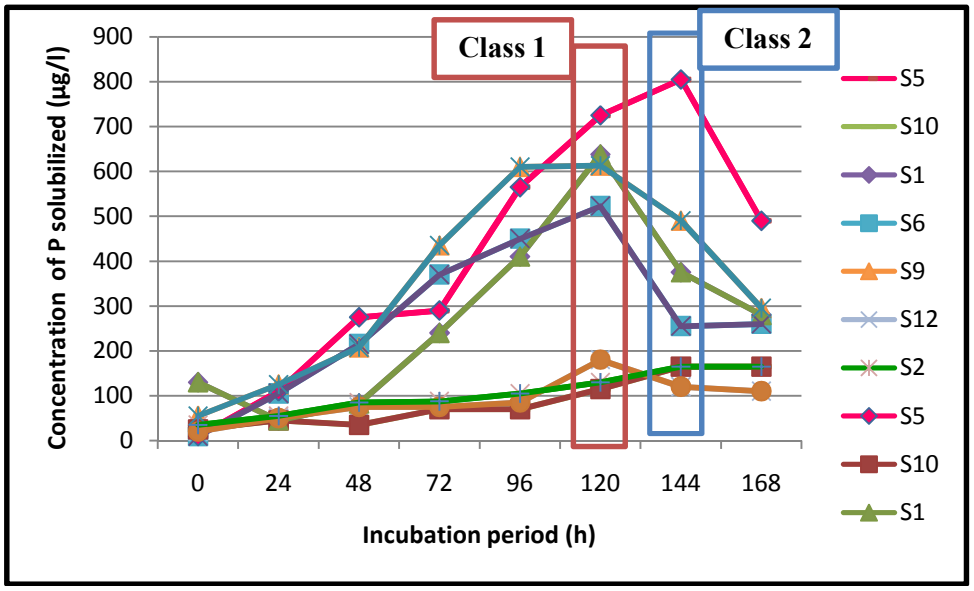


**Fig. 3. Class1: strains with a maximum rate of solubilization reached after 5 days of incubation; Class2: strains with a maximum solubilization obtained after 6 days of incubation**

This acidification of the medium could mainly be due to the secretion of organic acids during the solubilization of the insoluble form of phosphate.

However, when testing the correlation between the solubilization index and the maximum concentration of the solubilized phosphorus, there was no relationship between these two parameters (r = -0.08). This result could also be concluded from the Fig. 6 which shows a non-linear dispersion of the different points. Also it's noticed from the results that the strains S2 & S12having high indexes of solubilization were characterized by a low solubilization of the phosphate in the broth medium (3.47 and 3.88 µg/ml respectively) compared to other strains; furthermore, the third strain (S6) which had the third significant index of solubilization was also characterized by an average solubilization in the broth medium with about 5.92 µg/ml. However,

the remaining strains tested were characterized by an average index of solubilization and a good solubilization in the medium. This good solubilization in the broth medium could be attributed to the good oxygenation provided by the stirring that also promotes a greatest multiplication of the strains.

### 3.4 Phenotypic Characterization

The qualitative assessment of the different phenotypic traits allowed deducing that there was a phenotypic diversity between the strains studied and it overlooked preference for using different carbohydrates as well as their ability to ferment or produce some metabolites (i.e. precipitation of $H_2S$ or gas production). However, all the tested strains were characterized by a rapid growth with a generation period estimated to be between 2 and 4 hours depending on the tested strain.

The results showed that none of the strains transplanted on the Kligler Hajna growth medium produced a black precipitate (iron sulfide), whereas a detachment of the agar was observed in the case of strains S5, S6 and S9, which showed a gas production due to the consumption of the carbohydrate from the growth medium.

Except for the strains 5 and 6, all the others tested strains were mobile; whereas only the strain 10 was able to use the citrate as a sole carbon source.

Generally, all strains tolerated acidity up to pH = 5 but none was developed in pH = 4. While they tolerated strongly alkaline conditions even those with pH = 11 (100% of the strains studied). The best growth of all strains was observed at pH = 7.

All the isolates were able to tolerate salinity even at values of 4 and 5% of NaCl despite that the isolates had a low growth at 5% NaCl.

The growth of the present phosphate solubilizing bacteria varied also with the temperature noting that the optimal temperature was 28°C. Except for the strain 6 which did not develop at a temperature of 4°C, all the other isolates grew. All the strains grew even at a temperature of 40°C. However, they could not grow beyond 53°C.

All the phenotypic parameters evaluated are summarized in Table 1:

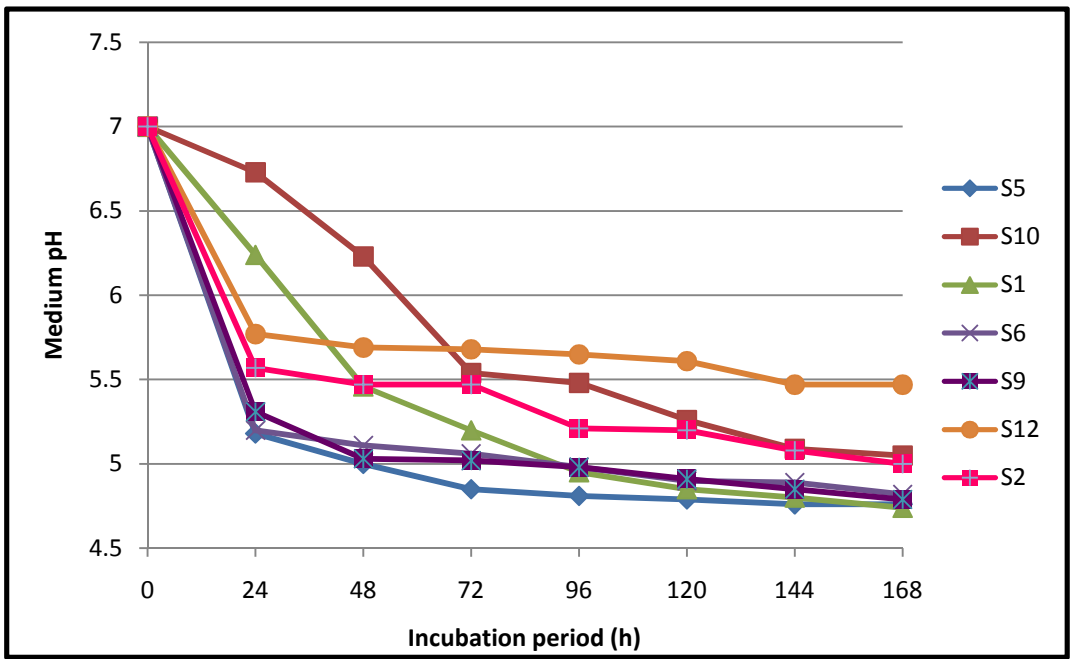


**Fig. 4. Variation of the medium pH with the incubation period and to the strain applied**

**Table 1. Biochemical characteristics of the solubilizing phosphorus bacteria selected**

| | | S1 | S2 | S5 | S6 | S9 | S10 | S12 |
|---|---|---|---|---|---|---|---|---|
| **Precipitation of $H_2S$** | | - | - | - | - | - | - | - |
| **Gas production** | | | - | + | + | + | - | - |
| **Motility** | | + | + | - | - | + | + | + |
| **Simmon citrate test** | | - | - | - | - | - | + | - |
| **Utilisation of carbohydrates** | Strach | + | ± | ± | ± | ± | ++ | ++ |
| | Arabinose | ± | + | + | ± | + | + | + |
| | Glucose | ± | ± | ± | ± | ± | ± | ± |
| | lactose | + | - | + | - | + | - | + |
| | Maltose | ++ | + | + | + | ± | ++ | ++ |
| | Mannitol | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| | Mannose | ± | + | | ± | + | + | ++ |
| | Sucrose | ++ | ++ | + | | ++ | ++ | ++ |

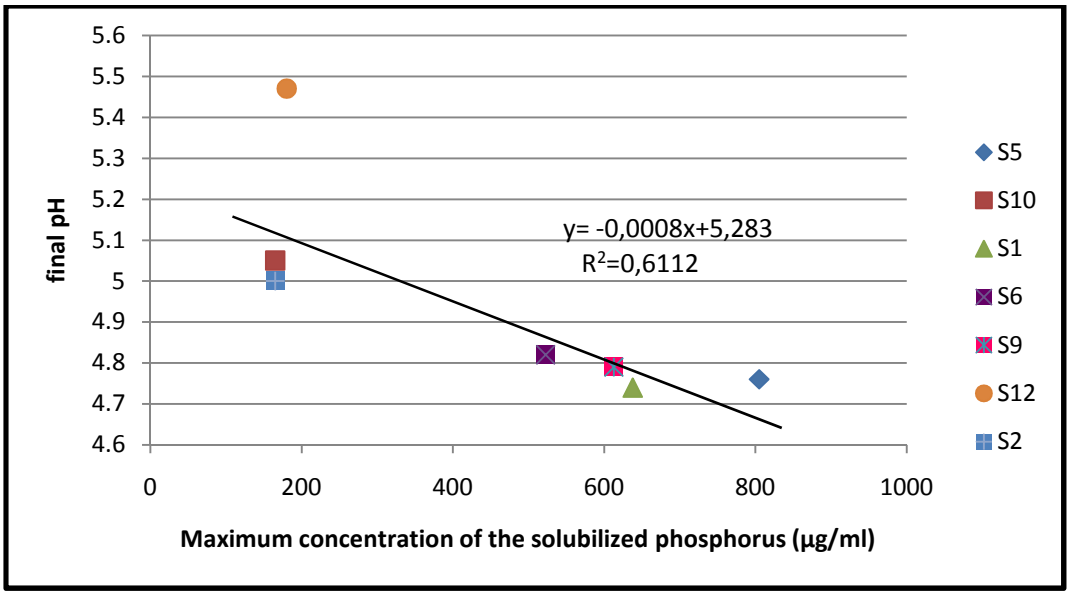


**Fig. 5. Relationship between the final pH of the growth medium and the maximum concentration of solubilized phosphorus**

## 3.5 Genotypic Characterization

According to the expected size of the16S rRNA gene [17] and for each PSB, the 16S rDNA was amplified in order to get a band of 1500 bp. The isolates were subjected to 16S rDNA gene sequence analysis. For our isolated strains, the 16S rDNA sequences determined in this study comprised 600 to 1500 nucleotides. There are widely accepted criteria for delineating species in current bacteriology, stating that strains with a sequence similarity greater than or equal to 97% may be considered a genus level match. A species level match is based on a similarity greater than or equal to 99% [18].

The 16S rDNA gene sequence analysis showed that there was a high similarity (≥ 99%) between four of the tested strains and their closest phylogenetic relative, which could indicate that the 16S rDNA genes sequence data were useful for the identification of isolates at the species level while the other three strains had percentages of similarity lower than 99% which could indicate just the level of genus. The strains that were identified to the species level were the strain PSB 1 which showed 99.6% of similarity with *Pseudomonas zeshuii*, the strain PSB 2 with 99.4% as a percentage of similarity with *Klebsiella oxytoca*. The strain PSB 9 had a percentage of similarity of 99.6% with *Pseudomonas frederiksbergensis* and finally the strain PSB 12 had 99.7% of similarity with *Citrobacter youngae*.

**Table 2. Phenotypic characteristics of the solubilizing phosphorus bacteria selected under environmental stresses**

| | | S1 | S2 | S5 | S6 | S9 | S10 | S12 |
|---|---|---|---|---|---|---|---|---|
| **Temperature (°C)** | 4 | + | ± | ± | - | ± | + | + |
| | 28 | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| | 40 | + | ± | ± | ± | ± | ± | ++ |
| | 53 | - | - | - | - | - | - | - |
| **(%) NaCl** | 0 | ++ | ++ | ++ | ± | ++ | ++ | ++ |
| | 1 | + | + | + | + | + | + | + |
| | 2 | + | + | + | + | + | + | + |
| | 3 | + | + | + | + | + | + | + |
| | 4 | + | + | + | + | + | ± | + |
| | 5 | ± | ± | ± | ± | ± | ± | ± |
| **pH** | 4 | - | - | - | - | - | - | - |
| | 5 | + | + | ± | + | + | + | + |
| | 7 | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| | 9 | ++ | ++ | ± | + | ++ | ++ | ++ |
| | 11 | ++ | ++ | + | ± | ++ | ++ | ++ |

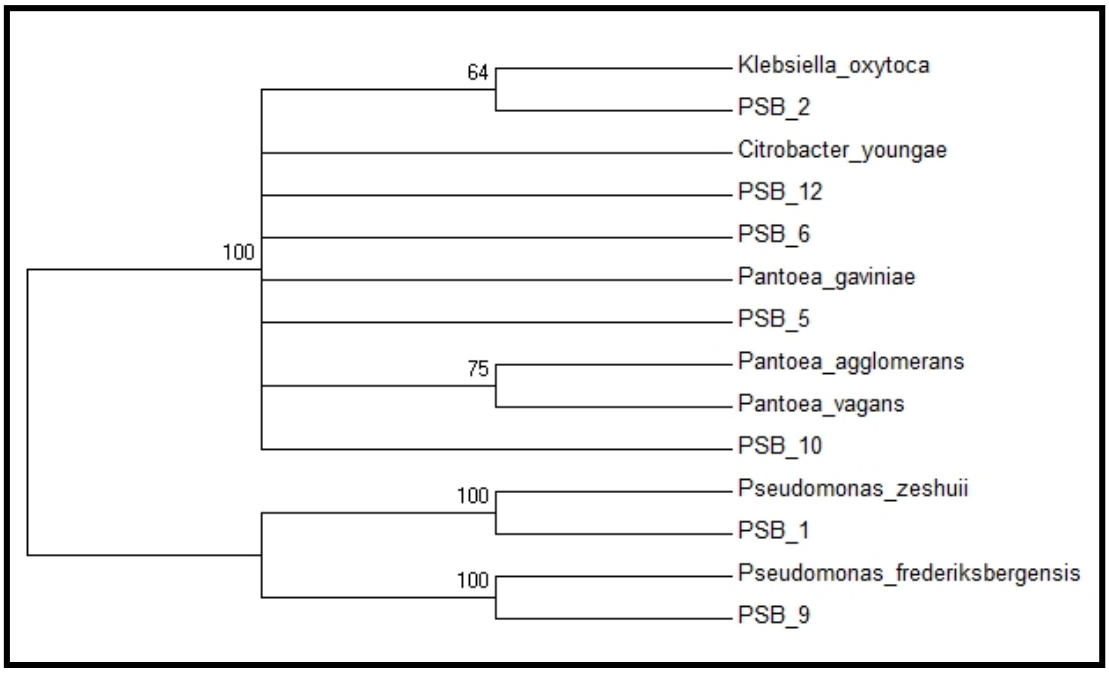


**Fig. 6. Neighbor-joining phylogenetic analysis of 16S rDNA sequences of PSB's isolates compared with the sequence of standard strains. Bootstrap probabilities were indicated and the bar represents a 0.02% of nucleotide divergence**

However the strains PSB 5 and PSB 6 had the same percentage of similarity 98.7% with *Pantoea agglomerans* and *Pantoea vagans* respectively. Finally the strain PSB 10 had 98.4% as similarity percentage with *Pantoea gaviniae*.

These results indicate that the identification of three of the characterized strains had reached just the genus level and since it's unable to get to the species level it could indicate that they may present new species. Based on these results, the isolated bacteria present genetically great diversity as they belong to different bacterial species.

## 3.6 Phylogenetic Analysis

Partial sequences of 16SrDNA of the different PSB's tested were used to construct phylogenetic tree using sequences of 16S rDNA

available from EzTaxon. The different PSB's sequences were compared to seven standard strains belonging to different species. Phylogenetic analyses with Mega 6 software indicated that strains PSB1, PSB6, PSB9 and PSB12 belonged with 100% bootstrap probability to *Pseudomonas zeshuii*, *Pantoea gaviniae, Pseudomonas frederiksbergensis* and *Citrobacter youngae* respectively.

However the strain PSB 2 belonged to *Klebsiella oxytoca* with 66% bootstrap probability, the PSB 5 and PSB 10 belonged with 75% bootstrap probability with *Pantoea agglomerans* and *Pantoea vagans* respectively.

## 4. DISCUSSION

The present study is the first characterization of the strains isolated from different sites in the Errachidia's province and having the potential to solubilize the TCP.

Generally, the effectiveness of the use of phosphorus through the application of phosphorus fertilizers is low due to the formation of the insoluble complexes [17]. Therefore, the frequent application of the soluble forms of the inorganic phosphorus is necessary for the agricultural production, however, this may increase the risk of the eutrophication of the aquatic systems [19].

Currently, there is great interest of the environmental concerns and sustainable development. Thus, numerous and various efforts are focused in order to develop practices that involve the use of techniques which are cheapest and from natural origin such as the application of phosphate solubilizing bacteria to improve the agronomic effectiveness [20,21].

Through this study, 19.4% of the 62 strains isolated from the province of Errachidia were selected for their phosphate solubilizing potential. Through the study of the phenotypic traits, heterogeneity was observed among the studied isolates. This heterogeneity was also confirmed at the genetic level since the isolates belong to different species and genus.

The selected strains showed different indexes of solubilizing on the NBRIP growth medium; a significant difference was observed between the solubilizing index of the first and the second measurements for the strains S12, S2, S5 and S1. Whereas, for the solubilizing index of the other strains, the difference was not significant between the first and the second assessments. Thus the results showed that as the diameter of the halo was important as solubilization index was high. This confirmed the results obtained by Baig et al. [22] and Yang et al. [23].

In the present even that some strains showed low solubilization indexes which still more important compared to other species already studied [16].

And based on the results of the solubilization indexes, seven strains were selected for the quantification of the solubilized phosphate on the NBRIP broth medium containing the TCP as sole source of the phosphate.

It was noticed that the concentration of the solubilized phosphorus differed from one strain to another and this result was in accordance to those of Leonardo [24] and Rfaki [25]. And no correlation was demonstrated between the index of solubilization and the concentration ($r = -0.08$) and this result confirmed those reported by Baig [22] and Yang [23].

However, as it had been reported by Chen [21] and confirmed by Asuming-Brempong [26], a negative correlation moderately strong was noticed between the final pH of the growth medium and the concentration of the solubilized phosphate. Thus, this acidification of the medium may be explained by the fact that the strains secreted and released organic acids [24,25] into the medium which promoted the solubilization of the inorganic forms of the phosphate and released the soluble form. Upon reaching the maximum solubilization, two cases were noticed; the first phenomenon was the reduction of the concentration of the solubilized phosphorus in the medium which could be explained by the fact that these strains used this solubilized phosphorus as a nutrient for their growth. For the second case a stabilization of the maximum concentration of the solubilized phosphorus was observed; in this case we cannot conclude if these strains used or not the solubilized phosphorus since we had stopped the study at the seventh day.

## 5. CONCLUSION

This first characterization had allowed to select 19,4% of the strains isolated from different sites of the Errachidia province of Morocco and featured by their solubilizing potential of TCP.

These bacteria selected have showed phenotypic heterogeneity confirmed by the genetic diversity. The qualitative evaluation of the selected isolates permitted to conclude that all of the selected strains have significant SI compared to anterior studies concerning other species of the PSBs.

The quantitative assessment allowed to deduce that the concentration of the solubilized phosphorus differs from one strain to another. However all strains showed a good solubilization compared to other species and genus already studied. Finally these studied strains can be a good organic alternative to promote agricultural production while respecting and preserving the environmental balance

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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