Isolation and biological characterization of phosphate solubilizing bacteria strains from the rhizospheric soils of corn in Mali

L'isolement et la caractérisation biologique des souches de bactéries du sol rhizosphérique de maïs solubilisant le phosphate au Mali

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Abstract

Forty-eight bacteria isolates from the rhizosphere of three cultivars of corn and three different soils were examined for their phosphate solubiling ability. The tests were realized on NBRIP (National Botanical Research Institute of Phosphate Growth medium) containing the Tilemsi phosphate rock (TPR) as the only source of insoluble phosphorus. All the isolates solubilized the TPR in solid and liquid media. Twenty isolates were selected for their high solubilizing capacity in liquid medium (105 to 311 mg P/ml/g of TPR). These isolates were submitted to different stress conditions (acid medium with pH 7 to 5, five successive growths and the temperature range of 30°C to 45°C). Only six isolates (I_1, I_2, I_3, I_4 I_5 ad I_6) were able to maintain their ability of solubilizing the TPR and also they were not antagonistics. These selected isolates were also tested for their solubilization efficiency (SE) of TPR and phytate on NBRIP solid medium containing the TPR and phytate as the only insoluble source of phosphorus and phytate respectively. The maximum solubilization (300%) for TPR was obtained with the isolate I_5 and 167% for phytate with the isolate I_1. It has been observed that the bacteria isolates have an average solubilization capacity of 18.54 kg P_2O_5 out off 30 kg contained in 100 kg of PNT. The same bacteria strains were tested for their plant growth promotion (PGPR) characteristics which indicated that all produced low molecular weight Organic Acids, Siderophores, Indole Acetic Acid (IAA or auxine), but none of them produced the Hydrocyanic Acid (HCN).

Keywords: Phosphate solubiliztion, Bacteria strains, rhizosphere, PGPR, corn.

Résumé

Quarante huit (48) souches de bactéries isolées de la rhizosphère de trois (3) variétés différentes de maïs et de sol ont été testées pour leur habilité à dissoudre les phosphates naturels. Les tests ont été réalisés sur le milieu de culture NBRIP (*National Botanical Research Institute of Phosphate Growth medium*) contenant du Phosphate Naturel de Tilemsi (PNT) comme seule source de phosphore insoluble. Tous les isolats ont solubilisé le PNT en milieux solide et liquide. Vingt (20) isolats ont été sélectionnés pour leur grande capacité de solubilisation en milieu liquide qui varie de 105 à 311 mg de P/ml/g de PNT. Ces isolats ont été soumis à différentes conditions de stress (milieux acides de pH 7, 6, et 5) ; 5 repiquages successifs et à des températures de 30°, 35°, 40° et 45°C). Seulement 6 souches bactéries (I_1, I_2, I_3, I_4, I_5, I_6) étaient capables de maintenir leur capacité de solubilisation du PNT et elles n'étaient pas

antagonistes. Ces isolats sélectionnés ont été testés aussi pour leur efficacité de solubilisation (ES) du PNT et du phytate en milieu solide contenant du PNT et du phytate comme seules sources insolubles de phosphore. L'efficacité maximale de solubilisation du PNT (300 %) a été obtenue avec l'isolat I_5 et 167 % du phytate avec l'isolat I_1. Il a été observé également que les isolats des souches bactériennes ont une capacité moyenne de dissolution de 18 kg de P_2O_5 sur les 30 kg contenus dans 100 kg de PNT. Les mêmes souches bactériennes ont été testées pour leurs caractéristiques de production de substances favorisant la croissance de la plante. Ces tests ont révélé que toutes les souches produisent des acides organiques de faible poids moléculaire comme les siderophores et de l'acide indole acétique (AIA ou l'auxine naturel) et qu'aucune ne produit de l'acide cyanhydrique.

Mots clés : solubilisation du phosphate, souches de bactéries, rhizosphère, PGPR, maïs.

I. Introduction

Phosphorus, widely distributed in nature in both organic and inorganic forms, are not readily available to plants in a bound state. Many soil bacteria are reported to solubilize these insoluble phosphates through various processes (Seshadri *et al.*, 2000). Microorganisms capable of producing a halo/clear zone due to solubilization by organic acids in the surrounding medium (Das, 1989; Singal *et al.*, 1991) are selected as potential phosphate solubilizers and are routinely screened in the laboratory by a plate essay method (Gerretson, 1948) using either Pikovskaya agar (Pikovskaya, 1948) or Sperber agar (Sperber, 1958). Several reports on bacteria and fungi isolated from soil have evaluated their mineral phosphate solubilizing activity with various P sources such as calcium phosphate tribasic [$Ca_3(PO_4)_2$] (Illmer and Schimer, 1995), iron phosphate (Fe PO_4) (Jones *et al.*, 1991) and aluminium phosphate ($AlPO_4$) (Illmer *et al.*, 1995). An increase in P availability to plants through the inoculation of phosphate solubilizing bacteria has also been reported previously in pots experiments and under field conditions by the following researchers: Banik and Dey, 1981; Chabot *et al.*, 1996; deFreitas *et al.*, 1997 and Zaidi *et al.*, 2003. Availability of phosphate in soil is greatly enhanced trough microbial production of metabolites leading to lowering of pH and release of phosphate from organic and inorganic complex (Haque and Dave, 2005). Research results indicated that some heterotrophe microorganisms groups have shown their ability of solubilizing the inorganic forms of P by organic acids production which solubilize the phosphate minerals in soil solution (Halder *et al.*, 1990a, Gaur, 1990, Bojinova *et al.*, 1997 and He *et al.*, 2002). Among these important microorganisms and responsible for that dissolution, there are bacteries like: *Bacillus megaterium, B. subtilis, B. polymyxa, Pseudomonas straita* and certain fungi among which can be quoted: *Aspergillus awamori, Penicillum bilaii, P. digitatum and Trichoderma sp.*

Phosphate (P) deficiency in soil can severely limit plant growth productivity, particularly in legums where both the plants and their symbiotic bacteria are affected, and this may have a deterious effect on nodule formation, development and function (Alikhani *et al.*, 2006).

According to Antoun and Kloepper (2001), intensive interactions take place among the plant, soil, and soil microorganisms at the root zone. Plants up take most of their requirement in nutrients and water from the roots. They also release from the roots a large number of low molecular weight water soluble exudates such as amino acids, sugars and vitamins for the benefit of the soil microorganisms. Plant Growth-Promoting Rhizobacteria (PGPR) are bacteria that aggressively colonize plant roots. They can multiply and occupy the ecological niches found on plant roots, at most stages of plant growth. Their mechanisms of growth promotion are through biological control with production of organic acids, siderophore and similar products with very high affinity to ferric iron. The siderophores immobilize this essential element, inhibiting the growth of deleterious and soilborne bacterial or fungal

agents unable to use this iron complex. The direct plant growth promotion is done by the production of indole acetic acid (IAA). Inoculation of young maize plants with PGPR microorganisms had an action comparable to the application of IAA. Indole-acetic acid (IAA) is the most common natural auxin found in plants. IAA is involved in physiological processes such as cell elongation and tissue differentiation (Taiz and Zeiger, 1991). It has been associated with the plant growth promoting effect of numerous rhizospheric microorganisms (Patten and Glick, 2002; Persello-Cartieau *et al*., 2003; Vessey and Heisinger, 2001). In addition to its key role in the growth of plants, IAA plays an important role in numerous plant-pathogen interaction (Yamada, 1993; Jameson, 2000). These authors have also indicated that some PGPR can solubilize rock phosphate or the different poorly soluble inorganic forms of (P) in soil by the production of organic acids and acidic proton (H^+ ions). Phosphatase enzymes produced by PGPR also mineralize organic phosphate (P). By increasing the concentration of soluble phosphate in soil, phosphate solubilizing PGRP can improve plant phosphate nutrition and growth. Finally with phosphate-solubilizing PGPR, the increase in phosphate availability is probably not the sole plant growth promoting mechanism involved.

Experiments or studies were conducted in 2007. Six selected bacteria isolates from the rhizosphere of three cultivars of corn and three different soils were tested for their solubilization efficiency of the organic and inorganic phosphate and for their PGPR characteristics.

II. Material and methods

The rhizosphere soil samples collected were stored in cool aseptic conditions at the laboratory. These samples were diluted from 10^{-1} to 10^{-5}.

2.1. Isolation of the bacteria strains

An aliquot of 100 µl of the appropriate (10^{-1} to 10^{-5}) dilutions of the soils samples were used to inoculate Petri dishes containing NBRIP's Phosphate growth medium of Nautiyal, 1999), with TPR as sole source of phosphorus for the isolation of phosphate solubilizing bacteria (PSB) or with phytate for phytate solubilizing bacteria. The TPR was washed three times with hot water to remove the soluble P. The plates were incubated at room temperature (25°C) for 7 days, then the number of colonies distinguished by clear zone (halo) production, indicating the phosphate solubilization were determined, identified and sub-cultured (table 1). As Petri dishes assay is not considered a reliable method in determining a strain as phosphate solubilizer (Johri *et al.*, 1999), the pure cultures were further screened in NBRIP (Nautiyal, 1999) liquid containing TPR at a concentration of 5 g/l as insoluble P source. Finally the test for organic acids production and quantity of solubilized TPR by the isolates was done as follow: the culture supernatant was obtained by centrifugation and the test of

Table I. Bacteria colonies producing halos zones of TPR solubilisation.

Soil samples locations	Cultivars of corn	Bacteria colonies with halo zones
Kangaba	Dembanyuman	0
	Tiemantie	6
	Sotubaka	2
Bancoumana	Tiemantie	7
	Dembanyuma	6
	Sotubaka	8
Gouani	Tiemantie	4
	Dembanyuma	5
	Sotubaka	4
Total		48

Ascorbic Acid Method of Watanabe and Olsen, (1965) for determining phosphorus in water and $NaHCO_2$ was used. Phosphorus dosage was performed using the colorimetric method of Tandon *et al*. (1968). Also the relationship between the dissolved P and the quantity of acids produced by the isolates in the medium was established. The efficient isolates have been selected for further experiments.

2.2. Characterization of the bacteria strains

The bacteria isolates were submitted to different stress conditions (acids media; five successive growths and the temperature range of 30°C to 45°C) to determine their TPR solubilization efficiency. For acid medium, the test was done according to Witelaw (2000) and Tandon *et al*., (1968) where the isolates were tested at pH 7, 6 and 5. The isolates preserving their TPR solubilization capacity at pH 5 after 5 successive growths and at 45°C corresponding to the corn culture environment of Mali were selected. The antagonistic test among isolates was done by putting in contact two by two in a Petri dish containing TSA 15%. The antagonism appeared when the growth of one isolate is blocked by the other one. The contrary phenomenon indicated the non antagonism among isolates in contact.

The PGPR characteristics tests of the isolates were conducted using the methods of Schwyn and Neilands (1987), modified by Milagres *et al.* (1999) for Siderophore production; Bakker and Schippers (1987) for Hydrocyanic Acid (HCN) and John M. Bric *et al*. (1991) for Indole Acetic Acid production.

III. Results and Discussion

3.1. Soil samples

The physico-chemical characteristics of the soil samples are presented in table II. All 3 soils are poor in available phosphorus with the minimum in Gouani (1,68 kg/ha) compared to Kangaba and Bancoumana with 13,96 kg/ha and 8,92 kg/ha respectively. Iron was higher in kangaba than Bancoumana and Gouani which showed similar contents. The same tendency was observed for aluminium.

3.2. Isolated bacteria strains

The plate counting results of the corn cultivars rhizosphere bacteria colonies with binocular magnifying glass has shown 48 pure colonies presenting TPR solubilization halos as indicated in table II.

3.3. Biological characterization of bacteria strains

TPR and phytate solubilisation in solid medium

The halo formation due to the organic acids production by the microorganisms was illustrated by putting the Bromothymol blue reagent in the growth medium dish which in contact with the acids gave orange coloration surrounding the colony.

TPR solubilization in liquid medium

The 48 isolates tested and selected on solid medium were also tested in NBRIP liquid medium containing TPR as sole source of insoluble phosphorus source, using the colorimetric method of Tandon *et al.* (1968).

Table II. Physico-chemical characteristics of the soil samples

Sol	Texture	pH	C/N	MO (%)	Available nutrients items (kg/ha)					
					P	K	Ca	Mg	Fe	Al
B	Loamy-sand	4.8	8.50	0.29	8.92	113.57	456.96	120.96	26.36	0.4
G	Sand-loam	5.3	10.66	0.55	1.68	96.09	461.44	80.64	29.56	0.08
K	Sand-loam	5.0	13.66	0.72	13.96	87.36	734.72	59.14	61.92	0.10

B = Bancoumana; G = Gouani ; K = Kangaba

The general linear model (glm) analysis of variance (ANOVA) for solubilised P quantity has shown high significant differences among means at P<0.001 with a variation coefficient of 37.72% (table III).

Fischer protected LSD test allowed to classify the isolates in 6 homogeneous groups according to their TPR solubilization capacity in liquid medium. The first 20 isolates (table IV) having a solubilization capacity between 105 and 311 mg P/g TPR, were selected for the rest of the experiments.

Table III. Analyse of variance of solubilised P quantity (mg/g of TPR).

Source of variation	DF	Means square
Model	47	8550.18***
Error	46	1715.58
CV (%)		37.72

**** highly significant at P <0.001.*

Table IV. Classified means of TPR solubilized P quantities by the isolates, (LSD = 83,373).

Isolats	Means of solubilised P quantity (mg P/ml/g TPR)
I_1	310.90 A
I_{10}	264.10 AB
I_9	239.10 ABC
I_7	230.30 ABC
I_{11}	225.70 BCD
I_2	217.40 BCD
I_{12}	202.60 BCDE
I_3	173.70 EFCD
I_{13}	156.00 EFCDG
I_4	146.70 DEFGH
I_6	145.90 DEFGH
I_{14}	132.70 EFGHI
I_{15}	121.40 EFGHIJ
I_5	120.10 EFGHIJK
I_8	119.16 FGHIJK
I_{16}	117.92 FGHIJK
I_{17}	111.70 FGHIJKL
I_{18}	111.70 FGHIJKL
I_{19}	109.90 FGHIJKL
I_{20}	104.88 FFGHIJKLM

I=isolate; *the means followed by the same letter are not significantly different according to Fisher Protected LSD (P<0.05).*

TPR solubilization under different stress conditions

a. *Acid medium*

Among the 20 isolates submitted to different acidity conditions (pH 7, 6 and 5), 9 isolates have shown good performance in the 3 conditions (table V). The results in this table indicated that the isolates had some ability of TPR solubilization at pH 5 (acid medium).

Conserving TPR solubilization capacity by the microorganisms is an essential criterion to consider when choosing a bio-inoculum. This happened after successive growth of a particular isolate.

b. *Successive growth effect*

The results are indicated in table VI. The isolates having shown good performance in TPR solubilization during the 5 successive growths were selected. The dissolution/ solubilization halo diameter varies from 1.5 cm to 2.5 cm.

Table V. pH effect on TPR solubilization by the isolates (mg P/g TPR)

Soil samples	Isolates	Corn cultivars	P produced in mg/g TPR		
			pH 7	pH 6	pH 5
Gouani	I_5	Tiemantie	487.8	80.3	107.5
Gouani	I_9	Tiemantie	340.3	37.3	47.8
Bancoumana	I_1	Sotubaka	423.9	160.4	74.4
Gouani	I_2	Sotubaka	319.9	87.6	85.6
Kangaba	I_3	Tiemantie	346.5	79.7	86.4
Bancoumana	I_8	Sotubaka	253.7	63.4	63.6
Gouani	I_7	Dembanyuman	319.9	87.6	85.6
Gouani	I_4	Dembanyuman	374.7	87.1	82.2
Bancoumana	I_6	Sotubaka	360.8	86.9	83.4
BGK	Control	SDT*	157.6	54.4	51.2

*Sotubaka, Dembanyuman, Tiemantie; SD = Standard Deviation.

Table VI. Colonies diameter and TPR dissolution halo variation during 5 successive growths in Petri dishes.

Isolates		Successive growth times, diameter (cm) of colony & halo				
		1	2	3	4	5
I_5	Colony	0.5	1.0	1.5	1.5	1.5
	Halo	2.0	2.0	2.0	2.5	2.5
I_1	Colony	0.5	1.5	2.5	1.2	1.5
	Halo	1.5	2.0	3.5	2.0	2.5
I_2	Colony	0.8	1.0	0.9	1.0	1.0
	Halo	2.0	2.0	1.5	2.0	2.0
I_3	Colony	0.6	0.6	0.7	1.0	1.2
	Halo	1.0	1.0	1.5	1.5	1.5
I_4	Colony	0.6	0.8	0.9	1.0	1.0
	Halo	1.0	1.0	1.5	2.0	2.5
I_6	Colony	0.4	1.5	2.0	1.3	1.6
	Halo	1.5	1.8	2.0	2.5	2.3

c. *Temperature effect*

In table VII, the isolates have shown similar behaviour in their TPR solubilization activities. Good isolates were those which solubilized at the highest temperature (45°C). The 6 isolates having shown this good performance with 0,7 cm to 1,7 cm of dissolution halo diameter were retained for the rest of the experiments.

d. *Antagonism test between the selected isolates*

There was almost no antagonism between the isolates during the 7 days of incubation. The observed antagonism during the first two days for certain isolates I_2 & I_5 ; I_4 & I_1 ; I_4 & I_2 and I_5 & I_4 disappeared the following days. This indicated that these isolates were not antagonistics and they can be associated for an inoculum formulation.

The solubilization efficiency (SE) is determined as follow: SE (%) = growth diameter – colony diameter/colony diameter x 100 (table VIII).

In referring to the TPR solubilization capacity of the selected microorganisms in liquid medium the quantity of solubilized P_2O_5 was determined for each microorganism in 100 kg of PNT. Since 100 kg of PNT contain 30kg of P_2O_5 the quantity of P_2O_5 susceptible to be solubilized by each isolate is indicated in table IX.

Table VII. Colonies and TPR dissolution halo formation by the isolates at different temperatures.

Isolates		**Temperatures, diameter (cm) of colony & halo**			
		30°C	**35°C**	**40°C**	**45°C**
I_5	Colony	0.1	0.1	0.2	0.1
	Halo	0.6	0.5	1.1	0.7
I_1	Colony	0.2	0.2	0.3	0.2
	Halo	0.8	0.9	1.3	1.7
I_2	Colony	0.2	0.2	0.3	0.1
	Halo	0.9	0.8	0.8	1.0
I_3	Colony	0.2	0.1	0.2	0.4
	Halo	0.8	0.7	1.8	1.0
I_4	Colony	0.2	0.2	0.3	0.2
	Halo	0.7	0.7	0.9	0.9
I_6	Colony	0.2	0.3	0.5	0.4
	Halo	0.7	0.8	1.3	1.5

Table VIII. Solubilization efficiency (SE) of TPR and Phytate by the bacteria isolates in solid medium

Isolates	**Solubilization efficiency of TPR (%)**	**Solubilisation efficiency of Phytate (%)**
I_1	200	167
I_2	150	63
I_3	115	75
I_4	150	100
I_5	300	129
I_6	275	113

Table IX. Quantity of P_2O_5 solubilized by the selected microorganisms in liquid medium

Isolats	Quantity of solubilized P_2O_5 (kg/100 kg of PNT)
I_1	31.09
I_2	21.74
I_3	17.37
I_4	14.67
I_5	12.01
I_6	14.59
Average	**18.58**

The selection of microorganisms based on their capacity to dissolve the TPR and the produced soluble P quantity permitted to select 6 isolates that have been classified according to their efficiency of TPR dissolution in solid medium which varied from 114.29% to 300% of dissolved P. A study carried out on the *Pseudomonas fluorescens*, *Bacillus megateriums* and *Azospirillum* spp., by El-Komy (2005) *in vitro* solubilization of calcium phosphate got the values of 128% to 150%. Malaiah and Sridevi (2007) were able to show the evidence of tricalcic phosphate solubilization efficiency on bacto agar medium by 5 rhizobiums isolates among 46 and it varied from 33% to 150%.

In liquid medium, the dosage of the soluble phosphorus permitted to show the evidence of the phosphate dissolution capacity of the 6 selected isolates varying from 148.6 to 332.2 mg P/g of TPR. According to Babana and Antoun (2003), some bacteria may not have TPR dissolution capacity in solid medium but can have in liquid medium. Such a case was not observed since all relate isolates have dissolved in both solid medium and in liquid medium as well. It is necessary to signal that the Isolates I_5, I_6 and I_1 gave good results in liquid medium, while 1_2 and I_3 showed a satisfactory solubilization capacity in both conditions. It is important to note that isolated I_4 showed good solubilization ability in liquid medium. Johri *et al.*, (1999), Nautiyal (1999), Babana and Antoun (2003) and El-Komy (2005), have suggested that before selecting a microorganism as bio-inoculum it is always useful to test the solubilization ability in both mediums.

3.5. Organic acids produced and solubilized phosphorus

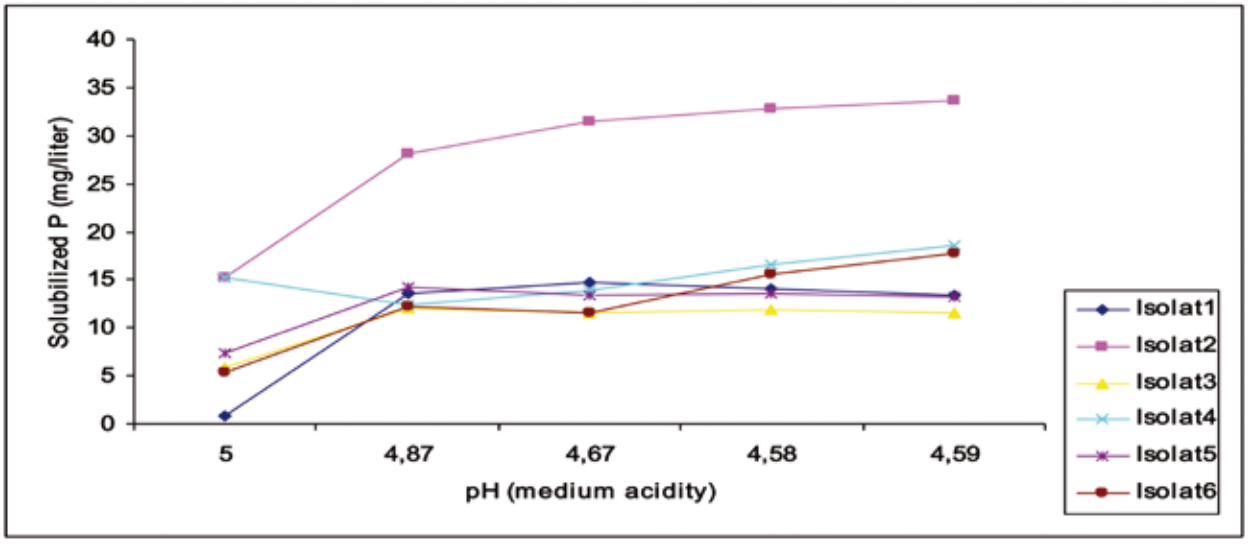
Graphic 1 shows TPR solubilization in relation to acids production or pH of the incubated solution (pH 5 to 4.59) obtained after 5 days of incubation. In this graphic, isolate I_2 has shown good TPR solubilization performance. The others showed relatively similar average performance trends (ie: I_1, I_3, I_4, I_5 and I_6).

According to graphic 2, the dissolved P was inversely proportional to the acids quantity produced or pH. Hence, the regression curve between the phosphorus dissolved and the acid produced by Isolate I_2 was represented by the following formula: $Y = a + bx$ or

$Y = 4.8552 - 0.7226x$. This negative correlation is the same for all the isolates where

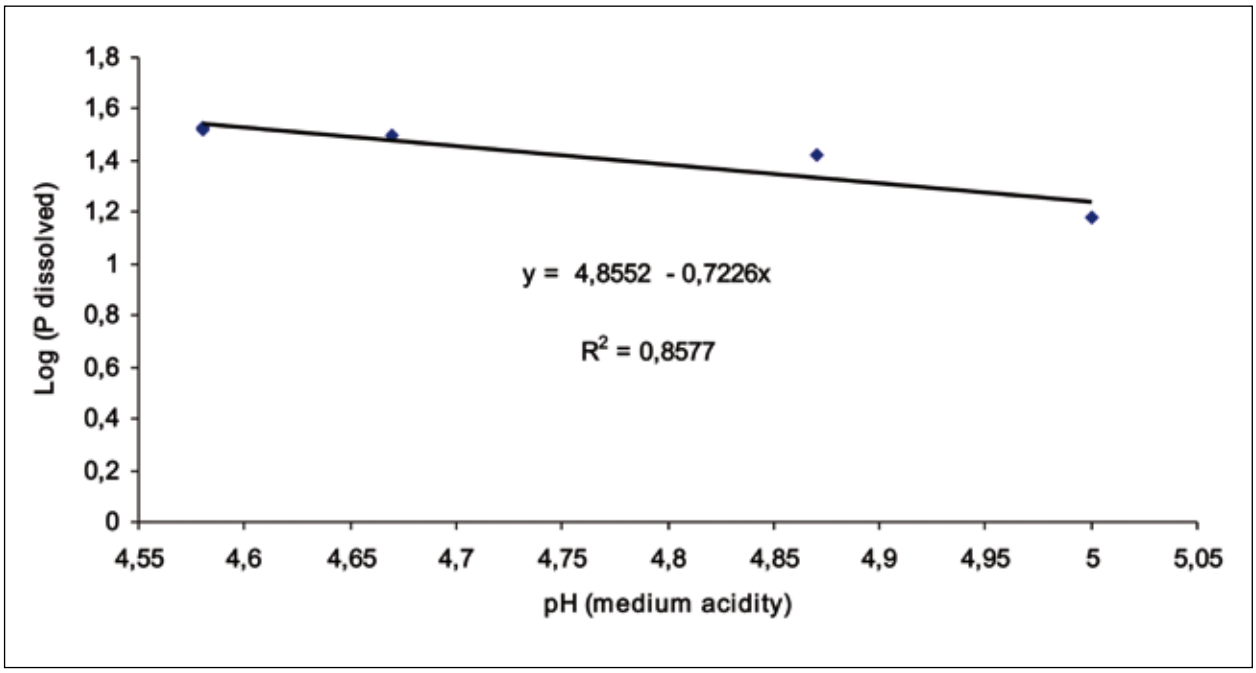
a = amplitude; b = correlation coefficient; x = pH and Y = regression curve

These results are in agreement with those obtained by Haque and Dave (2005) who indicated that the availability of phosphate in soil is greatly enhanced trough microbial production of metabolites leading to lowering of pH and release of phosphate from organic and inorganic complex. Halder *et al*. (1990a); Gaur (1990), Bojinova *et al*. (1997) and He



Graphic 1. Solubilized P as function of produced acids in the solution by the isolates.

d.



Graphic 2. Correlation between pH and the dissolved phosphorus by isolate $\mathbf{I_2}$.

et al. (2002), indicated that some heterotrophe microorganisms groups have shown their ability of solubilizing the inorganic forms of P by organic acids production which solubilize the phosphate minerals in soil solution. Among these microorganisms which are important and responsible for that dissolution, there are bacteria like: *Bacillus megaterium, B. subtilis, B. polymyxa, Pseudomonas straita* and some fungi such as *Aspergillus awamori, Penicillum bilaii, P. digitatum* and *Trichoderma* sp. Kucey *et al*. (1989) indicated from cultures supernatant certain numbers of organic acids such as: lactic acid, glycolic acid, citric acid, 2-ketogluconic acid, malic acid, oxalic acid, malonic acid, tartric acid and succinic acid. All have inorganic phosphates dissolution ability.

3.6. Growth enhancing products produced by the isolates

The PGPR characteristics of the isolates were illustrated by the production of:

a. *Produced Siderophores*

Siderophores production by the isolates (Schwyn and Neilands method (1987), modified by Milagres *et al*., 1999) was determined by measuring the average orange colour halo diameter surrounding the colony in the Petri dish. All the six isolates produced siderophores with an average diameter of the three measurements for each isolate indicated in table X. In fact, $I_{6;}$ $I_{5;}$ and I_1 were the most efficient.

b. *Hydrocyanic Acid (HCN) production*

According to the test of Bakker and Schippers (1987), none of the isolates produced HCN, because the test did not reveal any colour change from orange to grey colour indicating no HCN synthesis by the isolates.

c. *Produced Indole Acetic Acid (IAA)*

The indole acetic acid produced by the isolates was determined using the method of Bric *et al*. (1991). The reddish coloration surrounding the 2 mm colonies on the cellosic membrane under the Sarkovski reagent effect indicated the presence of IAA. The coloured bands diameters surrounding the colonies were measured and the average of 3 measurements for each isolate is indicated in table X. I_2, I_4, I_3 and I_5 have shown the highest production capacity.

It is necessary to mention that considering the reduced number of isolates, the diameters measurements for siderophore and indole acetic acid were not subject to any statistical analysis.

It has been observed from the different tests that all the isolates produced the siderophores and indole acetic acid. Dommergues *et al.*, (1999) reported that certain rhizosphere microorganisms produce vitamins such as thiamine, nicotinic acid, panthotenic acid and others which stimulate plants germination

Table X. Siderophore and Indole acetic acid produced by the isolates in Petri dishes

Isolates	Production diameter (cm)							
	Siderophores				Indole Acetic Acid			
	P_1	P_2	P_3	Means	P_1	P_2	P_3	Means
I_1	3.0	2.9	3.0	**2.96**	1.0	1.5	1.0	**1.16**
I_2	1.3	1.5	1.7	**1.5**	2.0	3.5	3.0	**2.83**
I_3	2.26	2.23	2.5	**2.33**	1.0	2.0	2.0	**1.66**
I_4	1.6	1.2	0.7	**1.66**	2.0	1.5	2.0	**1.83**
I_5	3.75	3.0	2.5	**3.08**	1.5	1.5	2.0	**1.66**
I_6	3.33	3.43	3.5	**3.42**	1.0	1.5	1.5	**1.33**

P = Petri dish

and growth. Rodriguez *et al*. (1998) indicated also that phosphorus deficiency increases the germination time, and the time between the first leaves emergence and tillering. The plants growth can be enhanced by certain soil microorganisms which colonized crops plants roots (Kloepper *et al*., 1999; Gray and Smith, 2005). Meanwhile plant growth regulation mechanism by the PGPR has not been yet sufficiently elucidated.

IV. Concluding comments

From the above results, it can be concluded that an interesting phenomenon of phosphates solubilization by these bacteria strains could be further studied to find out the mechanism of action or the involvement of genes. A study on the molecular mechanism would throw light on the phosphate solubilizing genes that could be incorporated in agriculture as bacteria strains offering traits for phosphate solubilization. Although no direct correlation could be established between *in vitro* solubilization of P, Plant P accumulation and available soil P, the results of this study make these isolates attractive as phosphate solubilizers. Also these bacteria are PGPR microorganisms by their ability of producing siderophores and indole acetic acid (IAA) which can give them the properties of improving plant phosphate nutrition and growth.

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