Original Research Article

Impact of Bacterial Isolates on Phosphate Amendment Solubilization in Rice Cultivation on Acidic Soils

ABSTRACT

This study was carried out in 2023 at the National Agronomic Research Center (CNRA) station in Man, in west of Côte d'Ivoire. The aim of this study was to improve the efficiency of Phosphate Solubilizing Bacteria (PSB) in the mineralisation of various phosphate amendments. Culture, isolation and purification tests on strains from the study site revealed the dominance of one strain. In the laboratory, it was associated with different treatments. The results show that it has a very highly significant effect on the parameters of the cultivation environment. The treatments increased pH. As the number of PSBs increased in the environment, dissolved P and Dissolved Organic Carbon (DOC) levels increased, while pH decreased over time. DOC levels were higher with inoculation (1.96 $mg.kg^{-1}$). The rate of mineralised P was highest (49.5 $mg.kg^{-1}$) on treatment T8 (0%PR (Phosphate Rock+100%TSP (Triple Superphosphate)) but treatment T5 (60% PR+40% TSP) would be the most appropriate for field trials; compared with day 1, it gave, on average, the highest rate of P released. The combination of PSB and PA would be a promising alternative for increasing the effectiveness of PA, particularly with regard to the use of Phosphate Rock (PR).

Key words: phosphate rock, Triple Super Phosphate, acid soil, Man, Ivory Coast.

INTRODUCTION

Like potassium (K) and nitrogen (N), phosphorus (P) is one of the three major nutrients involved in plant growth, but it is considered a limiting factor in soils, especially those that are both weathered and old in tropical regions [1]. According to Vance *and al* [2], 40% of the world's soils are deficient in P, especially those in tropical and subtropical zones. This deficiency in assimilable P leads to a drop in the yield and production of plants in general and rice in particular [3]. The best percentage of phosphorus available to the plant in the soil solution is between 2 and 5%.This lack of P that can be directly assimilated by the plant will limit the activity of microorganisms specialising in the mineralisation of soil organic matter, the content of which is also particularly low [4, 5]. To overcome this problem, soluble chemical fertilisers, which are prohibitively expensive for small-scale farmers, are applied to the soil to achieve appreciable yields. However, poorly applied phosphate fertilisers can reduce soil fertility by mobilising organic colloids [6, 7], and excessive use of mineral fertilizers leads to a reduction in nutrient use efficiency, especially phosphorus and nitrogen, with adverse effects on the atmosphere [8]. Also, according to Abbasi *et al.* [9], only 1% of applied phosphate fertilizers are used by the plant. According to Khan *et al* [10] and Servin [11], the majority (70-90%) of phosphate fertilizers used in agriculture precipitate after application. They form metal complexes that limit their effectiveness, which depends on soil

edaphic conditions such as CEC, type of cations, pH, humic substances and organometallic complex [12, 13, 11]. Thus, due to the high price of phosphate fertilizers, one of the low-cost alternatives is the use of phosphate rock (PR), which is a real source of phosphorus [3].

However, one of the main obstacles to the direct application of phosphate rocks to soils is the insufficient release of P to support plant nutrition due to their low solubility in the soil, which depends on soil characteristics [14, 11]. PSB's improve the amount of P solubilised in the soil and from phosphate amendments (PAs) in order to make it available to plants [10]. Also, the organic P contained in organic matter can only be available to plants after decomposition and mineralisation by microorganisms [15, 16].To increase the agronomic efficiency of phosphate rock (PR), several techniques have already been tested:

- composting organic residues with PR [17];

- partial acidulation of PR [18];

- mixing natural phosphates with water-soluble phosphate fertilizers [19];

- solubilisation of phosphate rock by microorganisms [11, 9].

However, phosphate fertilization must take into account the nutritional requirements of plants in terms of this element, but more importantly, it must take into account the different mechanisms by which it is made available in order to improve plant yields. With a view to improving the mineral nutrition of plants and remedying the drop in pH values, this work was carried out to assess the effectiveness of PSBs on the mineralisation of different types of phosphate soil improvers. The aim of this study is to assess the efficiency of PSBs on the mineralization of different types of phosphate amendments. Specifically, the aim is to determine the effect of phosphate amendments on the physico-chemical parameters of the environment and to assess the efficiency of phosphate solubilising bacteria (PSBs) in the various treatments.

MATERIALS AND METHODS

Study site

Our study site is located between 07°18' and 07°36' north latitude and 07°27' and 7°53' west longitude at the National Agronomic Research Center (CNRA) in Man station, in the west of Côte d'Ivoire. Soil samples were taken along the diagonals of the plot at 9 different points at a depth of 0-20 cm and mixed to obtain a representative composite sample of the site. The composite sample was divided into two parts. The first part was kept in the freezer for cultivation and counting microorganisms, and then for isolation of the phosphate-solubilizing bacterial strains. The second part was air-dried and used for physico-chemical characterization of the plot before the experiment was set up. The results of the physico-chemical characterization of the soil are shown in Table 2.

Chemical material

The phosphate rock from Morocco used consists mainly of CaO (49.54%) and P_2O_5 (30%). Its chemical composition is shown in Table 1.

Table 1: Physico-chemical characteristics of the soil in the 0 - 20 cm stratum before experimentation

Particulars	Values (0 – 20 cm)	Methods adopted	References
Physical properties			
Argile (%)	28.65	International pipette method	Piper [20]
Limon (%)	16.35		
Sable (%)	55		
Textural class			
Chemical properties			
pHeau 1 :2.5	5.2	pH meter	
Available P_2O_5 ($mg.kg^{-1}$)	5	Mehlich method	Mehlich [21]
Organic carbon (OC) (%)	1,43	Walkley and Black Method	
total Nitrogen (%)	0.13	Dry combustion - Elemental analysis (CHNO)	Bird et al., [22]
OM (%)	2,46	Calculated	
C/N	11	Calculated	
K^+ ($cmol^+.kg^{-1}$)	0.11	Helmke et Sparks Method	Helmke et Sparks, [23]
Na^+ ($cmol^+.kg^{-1}$)	0.11		
Ca^{++} ($cmol^+.kg^{-1}$)	1.00		
Mg^{++} ($cmol^+.kg^{-1}$)	0.32		
CEC	1.54	cobaltihexamine chloride absorbance	Aran et al., [24]
S/T (%)	18.23	Calculated	

Table 2 : Chemical elements of Moroccan Phosphate Rock

Chemical elements	**Values (%)**	Methods adopted	References
SiO_2	6.64	alkaline fusion	Singh and Gupta [25]
Al_2O_3	0.41		
Fe_2O_3	0.2		
CaO	49.54		
MgO	1.16		
P_2O_5	30		
Loss on ignition	12.05		

Biological material

The biological material consisted of a bacterial isolate from the soil at the study site. It was selected following isolation tests based on the characteristics described by Sharna et al, [26].

Isolation of strains

Isolation was carried out in three stages: pre-culture, selection and purification of bacterial strains on PVK medium.

Pre-culture consisted of using one hundred microlitres (100 µl) of the suspension from each dilution (10-1 to 10-6) of soil to inoculate microplates containing liquid PVK medium for 7 days of incubation. After incubation, a microplate reader was used to determine the number of positive wells, corresponding to the appearance of bacterial cloudiness, using a spectrophotometer to measure the OD at 620 nm. The results were processed by a statistical programme to determine the Most Probable Number (MPN) of bacteria present in the sample, expressed in CFU (Number of Colony Forming Units) per gram of dry soil. Selection was then made after 7 days incubation on solid PVK medium according to the morphological diversity of the strains. The morphological distinction of colonies was based on criteria described by Sharna et al [26]. These were colony shape, colony colour, colony appearance, colony diameter and solubilisation halos, followed by the solubilization index. The diameter of the colony and the surrounding halo are measured (Figure 1), then the solubilization index (SI) is calculated using the following formula:

$$IS = \frac{Colony\ diameter + Halo\ diameter}{Colony\ diameter}$$

Purification consisted of using isolates with an IS >2 to reseed petri dishes containing solid PVK medium (Figure 2). This procedure was repeated five (05) times until pure bacterial strains were obtained. The strain with the highest solubilization index was used to set up our experiment.

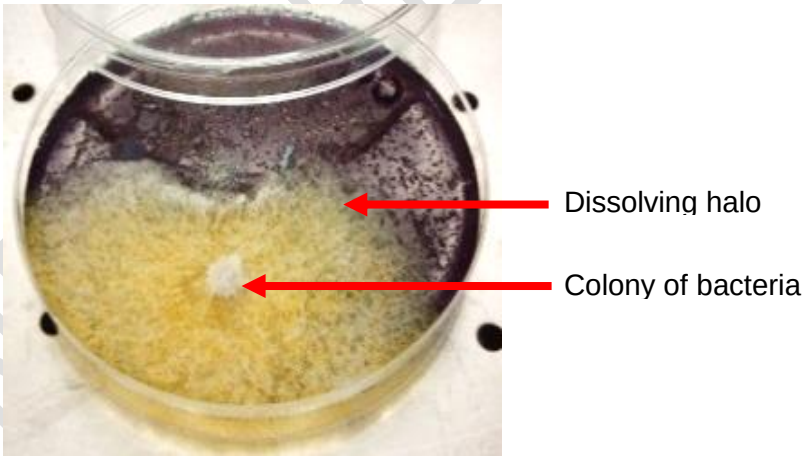

Figure 1 : inoculated microplate showing a halo of solubilization around a colony

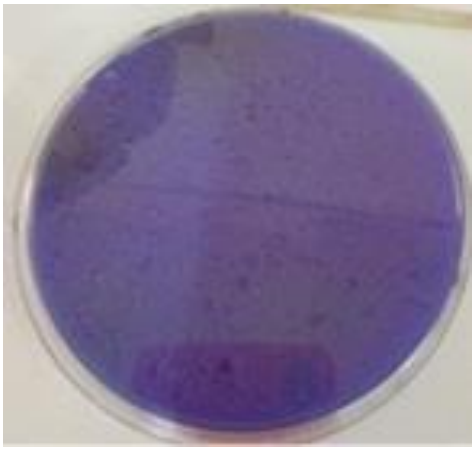

Figure 2 : Uninoculated control microplate

Testing the ability of bacterial isolates to solubilize phosphate in microcosms

The ability of bacterial isolates to solubilize phosphate was tested in microcosms in sterile 50ml polypropylene centrifuge tubes with tricalcium phosphate ($Ca_3PO_4^-$) or Moroccan phosphate rock (RP), previously UV sterilised, as the source of phosphorus. Thus, 40 ml of sterile PVK medium were placed in tubes containing either 2 g of tricalcium phosphate ($Ca_3PO_4^-$) or 3 g of Moroccan phosphate rock (to obtain 1 g/l of P in the medium) and 0.4 g of glucose (to obtain 10 $g.l^{-1}$ of glucose in the medium) was added to the PVK medium as the carbon source. The tubes containing the different PVK culture media were inoculated with 1 ml of isolated bacterial inoculum (10^8 bacteria/ml suspension). The tubes were shaken, wrapped in aluminium foil and incubated in the dark for ten (10) days. After 10 days of incubation, 5 ml of the different solutions were taken from each tube and centrifuged at 4,000 rpm for 15 minutes. The supernatant obtained was then used to determine the parameters (pH, P and number of bacteria) of the culture medium. Phosphorus was determined by colorimetry using a spectrophotometer at 790 nm. The number of bacteria was measured by spectrophotometer optical density at 620 nm.

Batch experiments

To complete the study on the ability of the selected bacterial strain to effectively solubilise phosphate, a batch experiment was carried out in the laboratory. In each 400 cm2 pot, 30g of sterile or non-sterile tray soil was introduced. Then 1.2g of phosphate amendments composed of different proportions of phosphate rock (PR) and/or triple super phosphate (TSP) to give 0.144g P/pot and finally 300 ml of sterilised distilled water to give a moisture content of 80% were added, then homogenised. The inoculated treatments received 250µl of bacterial isolate corresponding to 10^8 bacteria per pot. The jars were shaken and incubated for sixty (60) days. The phosphate amendments produced using different proportions of phosphate rock (PR) from Morocco and triple super phosphate (TSP) supplied by the Office Chérifien du Phosphate (OCP) are as follows: T0: 0% RP+ 0% TSP, T1: 100% RP + 0% TSP, T2: 90% RP + 10% TSP, T3: 80% RP + 20% TSP, T4: 60% RP+ 40% TSP, T5: 40% RP+ 60% TSP, T6: 20% RP+ 80% TSP, T7: 0% RP+ 100% TSP.

Data collection

After 0, 10, 20, 30, 40, 50 and 60 days of incubation, 5 ml of the different solutions were taken from each pot, centrifuged at 4,000 rpm for 15 minutes and the supernatant obtained was used to determine changes in the parameters (pH, P, dissolved organic carbon and number of bacteria) of the environment cultivation.

Statistical analysis

Using SAS 9.0 software, analyses of variance were carried out using the Student Newman_Keuls (SNK) test to determine the average rate of solubilised P, DOC, the number of bacteria and pH, per treatment at (*P = .05*)

RESULTS

Macroscopic characteristics of isolated strains

Overall, four (04) bacterial strains were identified on solid Pivovskaya (PVK) medium from the Plateau soil sample (Table 4). Macroscopic study of the bacterial strains showed that the diameters of the halo zone varied from 0.2 to 1 cm and the solubilisation index (SI) from 0.6 to 3.6 cm (Table 4). The colour of the strains identified is green-yellow (S1), brown (S2), brown-green (S3) and green (S4) and they have a rounded (S3 and S4) and subangular (S1 and S2) shape but they all have a cottony appearance. Furthermore, only the S1 strain obtained a solubilisation index IS>2, which is equal to 3.6 (Table 3).

Table 3 : Macroscopic characteristics of PSB

Isolates	Morphological characteristics	Halo diameter (cm)	SI
S1	Cotton aspect, green-yellow colour, subangular shape	1.03±0.15	3.6±1.2
S2	Cotton aspect, brown colour, subangular shape	0.2±0.01	0.6±0.04
S3	Cotton aspect, brown-green colour, rounded shape	0.7 ± 0.02	1.2 ±0.3
S4	Cotton aspect, green colour, rounded shape	0.4 ±0.03	1.5 ±0.5

SI = Solubilisation index

Effect of PA on environmental parameters

The addition of phosphate rock (PR) rich PA, i.e. 80 to 100% PR on inoculated soil and 60 to 100% PR on non-inoculated soil, significantly increased pH at *(P = .05)*, compared with treatments without PA. There was no significant difference between the pH determined under the treatment without PA and that under PA with a low proportion of PA (0 to 60% PA for the inoculated soil and 0 to 40% for the non-inoculated soil). The addition of phosphate amendment did not significantly modify bacterial proliferation at *(P = .05)*, whether the medium was inoculated or not, compared with treatments without PA. The addition of the phosphate amendment (PA) significantly increased the P content at *(P = .001)* compared with the treatments without PA, but this P content was highest when the proportion of PR in the phosphate amendment was 60% PR on non-inoculated soil and 0% PR on inoculated

soil. The greatest amount of solubilised P was observed in the treatments with 60% PR in the non-inoculated soil and 0% PR in the inoculated soil. With the exception of the treatment with 100% PR, the addition of AP significantly increased the amount of DOC in the medium at *(P = .05)* compared with the treatment without AP, but this increase was greater when AP contained 0 to 90% phosphate rock (PR) in the inoculated soil than in the non-inoculated soil (Table 4).

Table 4: Effect of phosphate amendments on environmental parameters after 60 days of incubation on non-inoculated soil

	Uninoculated				**inoculated**			
Treatments	**pH**	**PSB ($x10^8$)**	**P $mg.kg^{-1}$**	**DOC $mg.kg^{-1}$**	**pH**	**PSB ($x10^8$)**	**P $mg.kg^{-1}$**	**DOC $mg.kg^{-1}$**
without PA	4.9^{b}	3.65^{a}	17.22^{e}	1.19^{a}	4.9^{b}	3.65^{a}	17.22^{g}	1.25^{b}
100%PR	6.15^{a}	3.6^{a}	27.45^{d}	1.14^{a}	6.36^{a}	4.87^{a}	27.79^{f}	1.33^{b}
90%PR	6.14^{a}	3.65^{a}	26.57^{d}	0.91^{b}	6.13^{a}	4.93^{a}	31.5^{e}	1.60^{a}
80%PR	6.1^{a}	3.63^{a}	31.34^{b}	0.84^{b}	6.08^{a}	3.90^{a}	33.7^{d}	1.75^{a}
60%PR	6.12^{a}	3.6^{a}	36.92^{a}	0.64^{c}	5.24^{b}	4.91^{a}	32.18^{e}	1.50^{ab}
40%PR	5.32^{b}	3.64^{a}	26.46^{d}	0.64^{c}	5.19^{b}	4.96^{a}	34.74^{c}	1.79^{a}
20%PR	5.26^{b}	3.62^{a}	30.4^{b}	0.63^{c}	5.15^{b}	4.93^{a}	36.05^{b}	1.79^{a}
0%PR	5.21^{b}	3.62^{a}	28.47^{c}	0.54^{c}	5.10^{b}	4.94^{a}	40.63^{a}	1.76^{a}
CV (%)	5.41	18.18	2.04	10.12	5.53	16.53	1.77	8.04
Pr>F	.0001**	1 ns	.0001***	.0001*	.0001*	.2487 ns	.0001*	.0002**

*Data in the same column followed by the same letter are not significantly different according to the Student-Newman-Keuls test p<0.05. * very highly significant at the p<0.05 threshold; ** very highly significant at the p<0.05 threshold; ns: Not Significant.*

Effect of inoculation

With the exception of the 60% RP treatment, inoculation did not significantly increase pH. Inoculation lowered the pH (Figure 3). The addition of inoculum had no significant effect on the pH of the treatments.

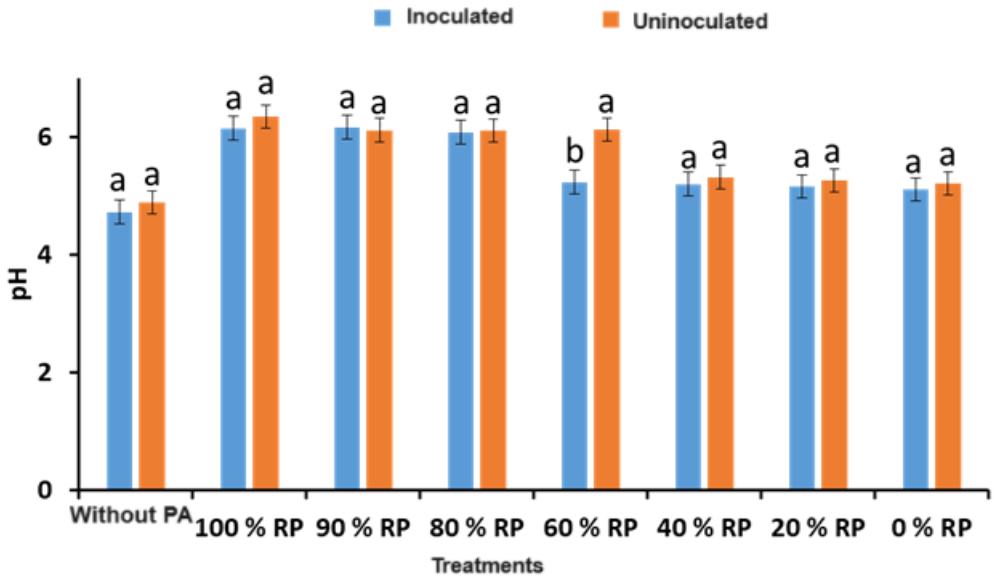

Figure 3 : Diagram showing how pH changes with different treatments according to soil type

The addition of inoculum significantly increased DOC levels at (*P=.05*), regardless of the treatment applied. In contrast to the inoculated soil, the DOC level in the media fell as the amount of PR in the treatments fell (Figure 4).

The addition of inoculum very significantly increased DOC in the inoculated soil treatments. This is particularly true when the proportion of PR in PA is low. In parallel with the quantity of PR in the treatments, the DOC level fell in the non-inoculated soil treatments.

The presence of inoculation significantly increases the rate of solubilised P, especially when PA is low in PR (0% to 40%PR). Inoculation had a significant effect (*P=.0001*) on the P content of the medium, especially in treatments with 90% PR and 60% PR, and DOC in all inoculated treatments compared with non-inoculated treatments (Figure 5).

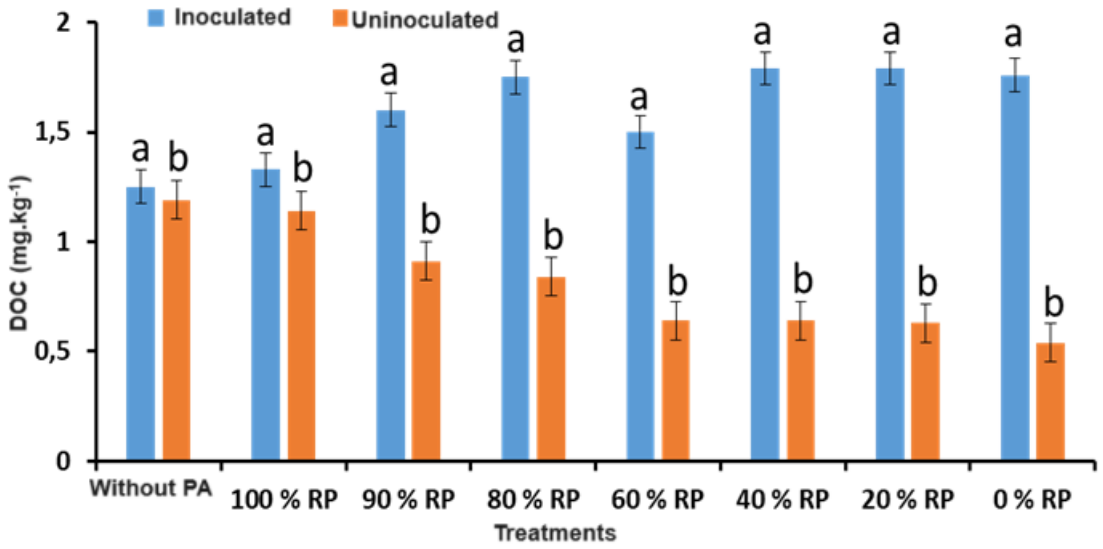

Figure 4 : Diagram showing Dissolved organic carbon (DOC) changes with different treatments according to soil type

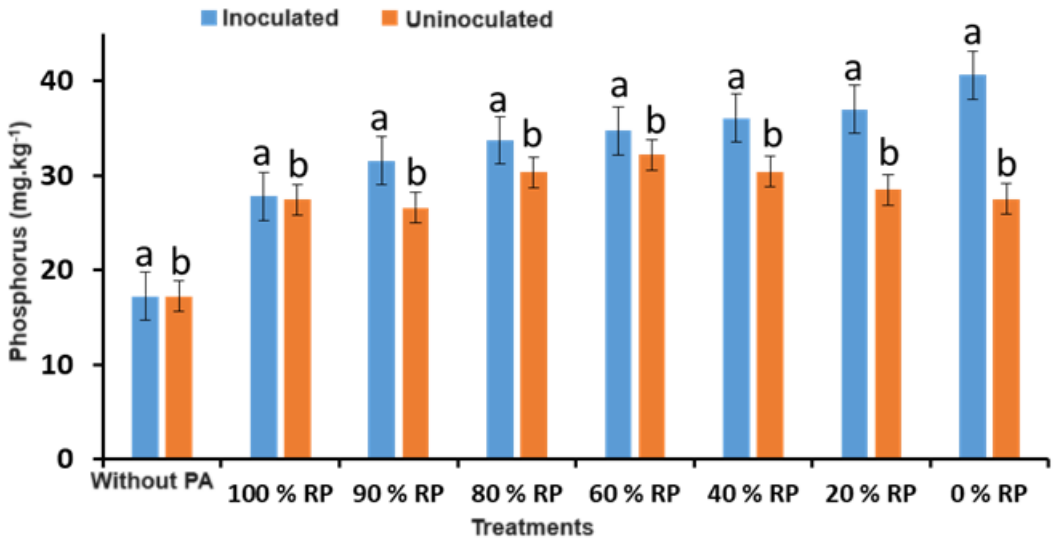

Figure 5: Diagram showing how Phosphorus levels changes with different treatments according to soil type

DISCUSSION

In fact, the appearance of a transparent halo (clear zone) around the growing colonies determines the solubilization of phosphate, due to the presence of acids produced by the BSPs. The SI of the strain (S1) is high, reflecting its high phosphate solubilization capacity (Table 4), as shown in the work of Hassimi et al. [27] and Haile et al. [28]. This selection method based on strain SI is consistent with that applied by Plassard et al., [29] who state that the ability to dissolve insoluble phosphate minerals supplied in a solid culture medium is a functional trait used to select so-called 'phosphate solubilizing' microorganisms.

Our results also showed that the higher the proportions of PR in the treatments, the higher the pH. The rapid increase in pH under treatments rich in PR is thought to be linked to the Ca

contained in this natural phosphate from Morocco (49% Ca), which, by binding to the clay-humus complex, reduces the concentration of H+ ions in the soil solution and consequently increases the pH of the medium [30]. This result confirm those obtained by Abbasi et al. [9], who showed that treatments with PR gave higher pH values than treatments with a soluble fertilizer (Simple Super Phosphate).The fact that the addition of a phosphate amendment does not significantly alter the proliferation of bacteria, whether the medium is inoculated or not, compared with treatments without PA could be due to indigenous soil bacteria.The P content of the different media, which varies according to the proportion of PA in the treatments, could be linked to the character of the two types of amendment. Natural phosphate rock is not very soluble in water, whereas triple super phosphate (TSP) is an easily soluble mineral fertilizer. Smalberger et al [15] have taken a similar approach, showing that the application of soluble phosphate fertilizers rapidly releases phosphorus to plants, whereas the application of PR alone releases phosphorus slowly, but its effect extends over several years.Treatment with 60% PR could be proposed for field trials to reduce the high cost of chemical inputs. The gradual fall in pH with inoculation is thought to be due to the activity of soil microorganisms, particularly MSPs, which, by decomposing organic matter or mineralising PA for their growth, secrete organic acids in the medium, thereby affecting pH [31, 32, 9]. The increase in DOC under the inoculated treatments is thought to be due to mineralization of the organic matter contained in the soil by the microorganisms, as demonstrated by the work of Bongoua-Devisme et al. [33], who observed a drop in soil pH and an increase in the number of bacteria after application of an organic amendment to rice field soils in Thailand, affected by salinity problems, probably due to the reductive Ferri activities of the bacteria. That said, the drop in DOC levels in uninoculated soil, when PR decreased in the treatments, would also be linked to a drop in the activity of the microorganisms. These results suggest that a combination of PSB phosphate solubilising bacteria and phosphate rocks could improve the reactivity of PR. This study shows that combining PSB-PR with soluble phosphate fertilisers could increase the effectiveness of the latter in order to increase the P available to plants.

CONCLUSION

The treatments applied affect the pH of the medium, the mineralised P and the DOC. The increasing rate of PSB favours the solubilisation of the PA present in the culture medium. PR applied alone increased the pH of the medium solution, whereas the phosphate chemical fertiliser used, TSP, acidified the medium but released more P when applied alone (T8). Inoculation had a highly significant effect on the parameters of the growing medium, as did the treatments applied. Although the treatments with TSP gave the highest levels of solubilised P, treatment T5 (60% PR + 40% TSP) would be appropriate for cultures in a real environment. In fact, compared with the first day, this treatment gave the highest average 'growth' rate. Combining PSBs with PAs is therefore a very promising alternative for increasing their effectiveness, particularly with regard to the use of PRs. However, field experiments would be necessary to confirm the profitability of this combination, both agronomically and economically, since Côte d'Ivoire does not produce the inputs used. It would also be advisable to identify the strains isolated from the soil sampled by means of more detailed studies such as molecular biology.

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