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# Solubilization of inorganic phosphates and plant growth promotion by strains of *Pseudomonas fluorescens* isolated from acidic soils of Cameroon

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**A trial of a screening and selection strategy for phosphate-solubilizing bacteria based on phosphate solubilization ability, and the subsequent effect of these strains on plant growth promotion under *in situ* conditions was conducted. Of all the bacteria tested, three *Pseudomonas fluorescens* strains (CB501, CD511 and CE509) were selected. On agar plates, two strains (CB501 and CE509) showed an ability to solubilize the three phosphate types ($Ca_3(PO_4)_2$, $AlPO_4 \cdot H_2O$ or $FePO_4 \cdot 2H_2O$), while strain CD511 showed a halo zone only on an agar plate supplemented with iron phosphate (Fe-P). However, in liquid media, all the strains were able to mobilize significant amounts of phosphorus (P) depending on the phosphate type. Calcium phosphate (Ca-P) solubilization resulted from the combined effects of pH decrease and carboxylic acids synthesis. At pH 4, it was solubilized by most of the organic acids. However, the synthesis of carboxylic acids was the main mechanism involved in the process of aluminium phosphate (Al-P) and Fe-P solubilization. Both were mobilized at pH 4 by citrate, malate, tartrate, and on a much lower level by gluconate and trans-aconitate. Subsequently, a greenhouse trial was conducted using *Zea mays*, the results of which obtained using 5 parameters including grain yield and P uptake, revealed that strain CB501 was the best plant growth promotor with a global effect of +37%, followed by strain CE509 (+21.2%) and then by strain CD511 (+16.7%). However, the selection of phosphate-solubilizing *Pseudomonas* strains as possible inoculation tools for phosphate-deficient soils should focus on the integral interpretation of laboratory assays, greenhouse experiments and field trials.**

**Key words:** Carboxylic acids, Phosphate solubilization, plant growth, *Pseudomonas fluorescens*.

## INTRODUCTION

Phosphorus (P) is one of the major essential macro-nutrients for biological growth and development (Fernandez et al., 2007). Most agricultural soils contain large reserves of total P, a part of the accumulated P depends on regular application of chemical fertilizers or sludge from wastewater treatment (Gyaneshwar et al., 2002; De-Bashan and Bashan, 2004). Both P fixation and precipitation occur in soil, because of the large reactivity of phosphate ions with numerous soil constituents (Rodriguez and Fraga, 1999; Fernandez et al., 2007). The concentration of soluble P in soil is usually very low (Goldstein, 1994) especially in ferralsols of sub Saharan Africa. This pool of immediately available P is extremely small and must be replenished regularly to meet plant requirements (Bieleski, 1973). P replenishment, particularly in subsistence agriculture, remains a challenge as it is mainly fertilizer-dependent. While the use of soluble mineral phosphate fertilizers is the obvious best means to combat phosphate deficiency in Cameroon, their use is

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**Table 1**. Soils chemical analysis of the investigated areas in Cameroon. The locations consists of oxisoils with poor total phosphorus (ranging from 140 - 210 mg·kg$^{-1}$) and very low availability as a result of high iron and aluminium contents. In general soils are acidic (pH 4 - 5).

| Provinces | Location | Strains | pH | Total P* | Available P | Total N(%) | Total C (%) | Fe* | Al* | Ca* | K* | Available P / Total P(%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | (mg·kg$^{-1}$) | | | | (mg·kg$^{-1}$) | | | | |
| Centre | Bokito | CB501 | 4.72 | 140 | 8.30 | 0.08 | 1.19 | 11530 | 13390 | 0.42 | 0.61 | 5.9 |
| | Douala | CD511 | 4.51 | 210 | 49.90 | 0.10 | 1.16 | 8070 | 9930 | 0.48 | 0.39 | 23.8 |
| Littoral | Edea | CE509 | 4.06 | 170 | 4.45 | 0.21 | 2.69 | 21270 | 3350 | 0.49 | 0.22 | 2.6 |

All data are mean of two replications; * aqua regia extract.

use is always limited by their high cost to the farmers. Interest has been focused on the inoculation of phosphate-solubilizing microorganisms into the soil so as to increase the availability of native, fixed P and to reduce the use of fertilizers (Illmer and Schinner, 1992). In particular, soil microorganisms are effective in releasing P from organic pools of total soil P by mineralization (Abd-Alla, 1994; Bishop et al., 1994) and from inorganic complexes through solubilization (Kucey et al., 1989; Richardson, 1994). Phosphate solubilization occurs by carboxylic acids synthesized and release by microorganisms; this released also decreases pH (Puente et al., 2004; Rodriguez et al., 2006).

Fluorescent *Pseudomonas* spp. are prevalent in the rhizosphere of plants (Lambert et al., 1987; Lamanceau et al., 1995). Certain members of this group are called plant growth-promoting rhizobacteria (Schroth and Hancock, 1982) because they are able to promote plant growth through solubilization of inorganic phosphates present in soil and through protection of plants against diseases caused by phytopathogenic fungi (Thomashow and Weller, 1988) . *Pseudomonas* spp. have been shown to be well adapted for growth and able to compete effectively for sites in the rhizosphere where nutrients are available (Bowen and Rovira, 1976; Kloepper et al., 1991). According to Dileep Kumar (1998), seed bacterization with these plant growth-promoting rhizobacteria for disease suppression and increased plant growth and yield is fast emerging as a potential field in plant biotechnology

The objective of the present study was to assess the efficiency of three *Pseudomonas fluorescens* strains in solubilizing sparingly soluble phosphates in laboratory media and by monitoring maize plant growth under greenhouse-limiting conditions.

## MATERIALS AND METHODS

### Isolation, purification and identification of microorganisms

Microorganisms were obtained from soil and root fragment samples collected in oil palm rhizospheres located in two agro-ecological zones of Cameroon, and representing as far as possible various levels of acidity, aluminium and iron toxicity. The areas investigated consist of three oxisoils with characteristics described in Table 1.

Soil samples were suspended in sterile distilled water, while root samples were macerated in sterile 0.85% NaCl solution. In both cases, serial decimal dilutions of the homogenate were prepared, individually plated on modified Bunt and Rovira agar medium (Fankem et al., 2006) and left to incubate three days at 28 - 30°C. Colonies surrounded with a halo zone were purified and kept at 4°C until use.

Purity testing was performed on agar plates containing nutrient agar with one of three sparingly soluble phosphates ($Ca_3(PO_4)_2$, $AlPO_4 \cdot H_2O$, $FePO_4 \cdot 2H_2O$) plus 0.5% Bromo-Cresol-Green as dye for better observation. Plates were inoculated with a 10 µl microbial suspension consisting of approximately 1 to 2 x $10^7$ colony forming units (CFU)·ml$^{-1}$ and left to incubate for five days at 28 – 30°C. The diameter of the colony (*n*) and that of the halo zone (*z*) were measured and the ratio *z/n* evaluated. Bacterial strains were identified by amplification of the 16S ribosomal DNA from pure microbial isolates, followed by partial sequencing of the amplicon (Fankem et al., 2006).

### Effect of pH and carboxylic acids on phosphate solubilization

An *in vitro* test was carried out to assess the P-mobilizing effect of pH, as well as the P-mobilizing effect of the identified carboxylic acids. Phosphorus was extracted with various HCl concentrations (0 - 20 mM) or NaOH (0 - 0.5 mM), or various concentrations of important carboxylic acids (0 - 10 mM). In the last case, the pH was adjusted to 4, as well as 7 to determine anion effect without acidification. The extraction was made by mixing 100 mg of either Ca-P, or Al-P or Fe- P in 30 ml of solution and stirring the mixture for 90 min at 150 rpm·min$^{-1}$. For each extrac-tion, duplicate flasks were used. The pH of the medium was measured before and after the experiment. At the end of the incubation period, the solution was centrifuged at 6000 × *g*, and the soluble phosphate in solution was measured (Murphy and Riley 1962), as described above.

### Greenhouse trials with phosphate-solubilizing *Pseudomonas fluorescens* strains

Greenhouse experiments were carried out in plastic pots filled with a mixture of sterilized soil (pH, 4.89; total P,

**Table 2.** Halo zone response of *P. fluorescens* strains on agar plates containing sparingly soluble phosphate types.

| Strains | Nutrient Agar with $Ca_3(PO_4)_2$ | | | Nutrient Agar with $AlPO_4 \cdot H_2O$ | | | Nutrient Agar with $FePO_4 \cdot 2H_2O$ | | | Rating |
|---|---|---|---|---|---|---|---|---|---|---|
| | z(mm) | n(mm) | z / n | z(mm) | n(mm) | z / n | z(mm) | n(mm) | z / n | |
| CB501 | 21.63 | 13.00 | 1.66 | 20.00 | 15.00 | 1.33 | 19.38 | 10.44 | 1.87 | abc |
| CD511 | 0.00 | 24.88 | — | 0.00 | 17.75 | — | 24.00 | 15.63 | 1.54 | c |
| CE509 | 29.75 | 12.75 | 2.33 | 17.63 | 10.38 | 1.70 | 23.50 | 12.38 | 1.90 | abc |

Data are means of two replications. Note: z = diameter of halo zone, n = diameter of colony. Rating: a = solubilization of $Ca_3(PO_4)_2$, b = solubilization of $AlPO_4 \cdot H_2O$, c = solubilization of $FePO_4 \cdot 2H_2O$.

**Table 3.** Phosphate solubilization activity and pH variation in liquid media amended with the three phosphate types recorded seven days after inoculation**.**

| Strains | Media supplemented with $Ca_3(PO_4)_2$ | | Media supplemented with $AlPO_4 \cdot H_2O$ | | Media supplemented with $FePO_4 \cdot 2H_2O$ | |
|---|---|---|---|---|---|---|
| | Solved Pi mg $l^{-1}$ | pH value | Solved Pi mg $l^{-1}$ | pH value | Solved Pi mg $l^{-1}$ | pH value |
| Control | 15.29 a | 6.27* | 29.88 a | 7.06* | 29.73 a | 6.72* |
| CB501 | 99.80 c | 5.00*** | 37.88 b | 4.02**** | 44.65 b | 4.32*** |
| CD511 | 59.46 b | 5.40** | 92.15 d | 5.11** | 47.59 c | 4.92** |
| CE509 | 58.56 b | 4.87*** | 56.94 c | 4.69*** | 51.01 d | 5.02** |

Note: Data are means from experiments performed in triplicate. For each phosphate source, data followed by different letters or stars are significantly different ($p < 0.05$) according to ANOVA test performed with SPSS 10.1 Software.

337. 44 mg·$kg^{-1}$; Bray P, 22.99 mg·$kg^{-1}$, P soluble in water,0.35 mg·$kg^{-1}$) and sand (3/1), at about 7 kg/pot. Sénégal rock phosphate (33 % $P_2O_5$) was added to the soil before cultivation at 0.3 g·$kg^{-1}$soil. Maize (*Zea mays* var. CMS8704) was selected because of its fast growth, with a short life cycle of 90-110 days. Seeds were sterilised with sodium hypochlorite (25 g·$l^{-1}$), washed and kept wetted overnight to provide suitable moisture for germination. They were then sown the next day and each seed bed was inoculated with 1 ml of a two day-old microbial culture containing approximately 1 to 2 x·$10^7$ CFU·$ml^{-1}$. Uninoculated control pots were also set up. All pots were irrigated after sowing and randomly deposited in a greenhouse subjected to ambient light and natural rainfall. In case of dryness, pots were supplied with tap water to avoid desiccation. Nitrogen was supplied in the form of a urea solution (33% N) at 20 g·$l^{-1}$ after 30 and 60 days of growth. The experiment included a control without inoculation and inoculated treatments (CB501, CD511 and CE509) performed in twelve replications. Growth characteristics in terms of plant height were evaluated 67 days after planting (DAP), while parameters such as shoot dry weight, plant yield, shoot and seed P content were evaluated at 117 DAP.

#### Statistical analysis

Data were statistically analyzed using means comparison and ANOVA tests performed with SPSS 10.1 Software (MARKINOR, Douala, Cameroon).

## RESULTS

### Activity of isolates on agar plates

The data in Table 2 indicate the values of colony diameter *(n)*, that of the halo zone *(z)* and the *z/n* ratio of the different strains obtained on agar plates containing different phosphate types. The ratio *z/n* helps to evaluate the activity of strains; the higher the value of the ratio, the greater the activity of the tested strain. The activity was associated with a pH decrease of the medium, observable through the yellow zone surrounding bacterial colonies. Among the three strains tested, only *P. fluorescens* CD511 did not show a halo zone on agar plates containing Ca-P and Al-P, respectively (Table 2).

### Activity of *Pseudomonas fluorescens* strains in liquid cultures containing sparingly soluble phosphates

Table 3 summarizes the total amounts of P released (mg P·$l^{-1}$) in liquid cultures and the final pH of the corres-ponding media at the end of incubation period from each *P. fluorescens* strain. The amount of P solubilized increased with time, and at the end of the incubation pe-riod (seventh day), the values obtained were significantly different from those of the control, irrespective of P source. This showed that the strains tested had effect-tively converted the inorganic insoluble phosphate into a soluble form. In the media supplemented with $Ca_3 (PO_4)_2$, the highest amount (99.80 mg P·$l^{-1}$) was obtained with strain *P. fluorescens* CB501 isolated from root fragments collected at Bokito in the centre province. In the media supplemented with $AlPO_4 \cdot H_2O$, the highest value (92.15 mg P·$l^{-1}$) was recorded with strain *P. fluorescens* CD511 isolated from root fragments, collected at Douala in the Littoral province, while in the medium supplemented with $FePO_4 \cdot 2H_2O$ as sole P source, the highest P release value (51.01 mg P·$l^{-1}$) was obtained with strain *P. fluore-scens* CE509 isolated from soil sample collected at Edea in the Littoral province. In general, the amount of phos-phate solubilized decreased in the order $Ca_3 (PO_4)_2$ > $AlPO_4 \cdot H_2O$ > $FePO_4 \cdot 2H_2O$. Phosphate solubilization was

**Table 4**. Concentration of organic acids produced by phosphate solubilizing *P. fluorescens* strains in nutrient solution at the end of incubation period.

| | $Ca_3(PO_4)_2$ | | $FePO_4 \cdot 2H_2O$ | | $AlPO_4 \cdot H_2O$ | |
|---|---|---|---|---|---|---|
| | $\mu g \cdot ml^{-1}$ | $\mu mol \cdot ml^{-1}$ | $\mu g \cdot ml^{-1}$ | $\mu mol \cdot ml^{-1}$ | $\mu g \cdot ml^{-1}$ | $\mu mol \cdot ml^{-1}$ |
| **Strain CB501** | | | | | | |
| oxalic acid | 0.00 | 0.00 | 1.84 | 0.02 | 0.00 | 0.00 |
| trans-aconitic acid | 25.05 | 0.14 | 21.75 | 0.12 | 23.33 | 0.13 |
| citric acid | 0.00 | 0.03 | 9.50 | 0.05 | 7.25 | 0.04 |
| tartaric acid | 0.00 | 0.00 | 59.42 | 0.40 | 0.00 | 0.00 |
| gluconic acid | 507.19 | 2.59 | 61.22 | 0.31 | 372.42 | 1.90 |
| malic acid | 101.82 | 0.76 | 370.38 | 2.76 | 198.61 | 1.48 |
| succinic acid | 5.18 | 0.04 | 53.99 | 0.46 | 38.92 | 0.33 |
| fumaric acid | 20.73 | 0.18 | 46.58 | 0.40 | 32.73 | 0.28 |
| **Strain CD511** | | | | | | |
| oxalic acid | 0.66 | 0.01 | 0.92 | 0.01 | 0.00 | 0.00 |
| trans-aconitic acid | 60.19 | 0.35 | 66.56 | 0.38 | 30.46 | 0.17 |
| citric acid | 45.75 | 0.24 | 105.57 | 0.73 | 20.11 | 0.10 |
| tartaric acid | 0.00 | 0.00 | 87.08 | 0.58 | 376.85 | 2.51 |
| gluconic acid | 654.57 | 3.34 | 139.82 | 0.71 | 0.00 | 0.00 |
| xylonic acid | 0.00 | 0.00 | 0.00 | 0.00 | 70.29 | 0.17 |
| malic acid | 0.00 | 0.00 | 78.12 | 0.58 | 0.00 | 0.00 |
| succinic acid | 0.00 | 0.00 | 11.66 | 0.10 | 16.89 | 0.14 |
| lactic acid | 14.91 | 0.17 | 0.00 | 0.00 | 0.00 | 0.00 |
| fumaric acid | 3.12 | 0.03 | 8.34 | 0.07 | 13.83 | 0.12 |
| **Strain CE509** | | | | | | |
| oxalic acid | 0.00 | 0.00 | 1.28 | 0.01 | 0.00 | 0.00 |
| trans-aconitic acid | 127.77 | 0.73 | 131.73 | 0.76 | 145.68 | 0.84 |
| oxalacetic acid | 3.52 | 0.03 | 4.53 | 0.03 | 7.92 | 0.06 |
| tartaric acid | 0.00 | 0.00 | 33.88 | 0.23 | 57.73 | 0.38 |
| gluconic acid | 2006.11 | 10.23 | 214.65 | 1.09 | 213.75 | 1.09 |
| malic acid | 23.83 | 0.18 | 82.43 | 0.61 | 35.63 | 0.27 |
| succinic acid | 147.43 | 1.25 | 62.48 | 0.53 | 79.51 | 0.67 |
| fumaric acid | 10.17 | 0.09 | 16.25 | 0.14 | 16.50 | 0.14 |

was associated with pH decrease of the media, but this pH decrease was not strictly proportional to the amount of the phosphate solved. For strains *P. fluorescens* CE509 (51.01 mg $P \cdot l^{-1}$) and *P. fluorescens* CD511 (47.59 mg $P \cdot l^{-1}$) the pH values are respectively 5.02 and 4.92 (not significantly different) (Table 3).

## Identification of carboxylic acids in nutrient solution

Various molecules such as oxalate, tartrate, succinate, trans -aconitate, gluconate, lactate, oxaloacetate, xylonate, fumarate, citrate and malate were purified in different media, and the type and concentration of molecules varied relatively from one strain to another and from one medium to another (Table 4). Among the eleven compounds, seven were produced by all the strains, while oxaloacetate and xylonate were exclusive to *P. fluorescens* CE509 and *P. fluorescens* CD511, respectively.

Lactate and citrate were produced only by *Pseudomonas fluorescens* CD511 and *P. fluorescens* CB501. When following the production of citric, tartaric, malic and gluconic acids in the different media, it appears that strains CD511 (59.46 mg $P \cdot l^{-1}$ Ca-P; 92.15 mg $P \cdot l^{-1}$ Al-P; 47.59 mg $P \cdot l^{-1}$ Fe-P) and CB501 (96.57 mg $P \cdot l^{-1}$ Ca-P; 93.96 mg $P \cdot l^{-1}$ Al-P; 50.73 mg $P \cdot l^{-1}$ Fe-P) were able to produce the four carboxylic acids in differing ratios, while strain CE509 (58.56 mg $P.l^{-1}$ Ca-P; 56.94 mg $P.l^{-1}$ Al-P; 51.01 mg $P.l^{-1}$ Fe-P) was not able to produce citric acid. Therefore, an influence of the type of carboxylate pro- duced on P solubilization is possible (Table 4).

## Effect pH on phosphate solubilization

The solubility of tertiary Ca-P is strongly influenced by pH (Figure 1); its solubility increased exponentially with decreasing pH (6 – 4). However, acidification reduces the

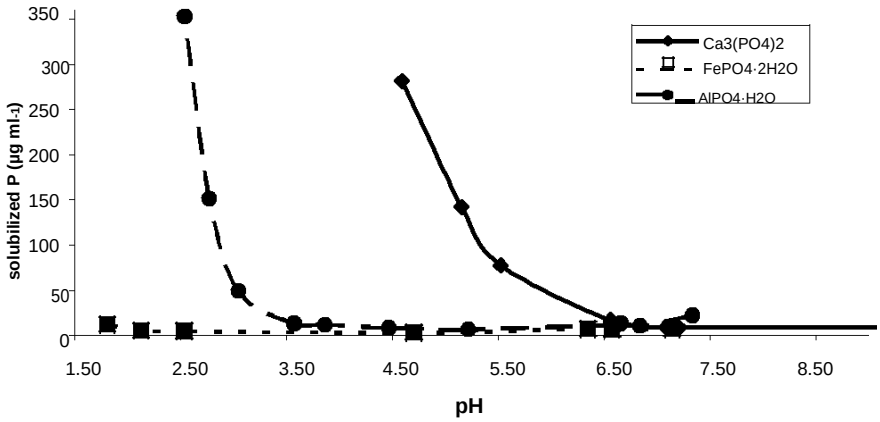


**Figure 1.** Solubilization of sparingly soluble phosphates as influenced by pH at the end of extraction.

**Table 5.** Effect of inoculation of phosphate-solubilizing *P. fluorescens* strains on the growth, phosphorus uptake and yield of maize under green house conditions.

| Strains | Plant height (cm) | Shoot dry weight (g) | Plant yield(g/plant) | Phosphorus uptake (µg) | |
|---|---|---|---|---|---|
| | | | | Shoot | Grain |
| Control | 155.10 a | 68.10 a | 56.30 a | 35.41 a | 16.37 a |
| CB501 | 187.70 c | 81.00 b | 76.50 c | 46.98 c | 28.90 b |
| CD511 | 181.00 b | 83.40 b | 66.20 b | 40.03 a | 18.56 a |
| CE511 | 190.20 c | 71.80 a | 58.70 a | 42.36 b | 25.17 b |

The tests of comparison of mean and ANOVA were performed using SPSS 10.1 software.
Values of each parameter followed by the same letter are not significantly different at $P < 0.05$.

bility of Fe-P (lowest solubility between pH 4.7 - 2.5 after shaking). Al- P showed the lowest solubility between pH 5.5 and 4.5. At pH 3.5, it was comparable with pH 7. Its solubility was also reduced at pH 5.2 with a strong increase at pH levels below 3 that are rarely significant in natural soils. Hence, acidification cannot be the explanation for phosphate mobilization in bacterial cultures in the last two cases (Figure 1).

## Effect of carboxylic acids on phosphate solubilization

The efficiency of carboxylic acids on P solubilization depends on pH. With respect to their natural occurrence, Ca-P extractions are effective at neutral pH, whereas Al-P and Fe-P should be mobilized under acidic conditions. Thus, the pH of the extraction solution was adjusted to a pH of natural occurrence of single phosphates (pH 7 for $Ca_3$ ($PO_4$)$_2$, pH 4 for $FePO_4$ and $AlPO_4$). The graphs (Figures 2a-d) allowed comparisons to be made regarding the efficiency of different carboxylates. Sparingly soluble Ca-P occurs only in neutral and alkaline soils. A strong pH decrease is impossible under well buffered soil conditions. At pH 7, only citrate solubilized significant amounts of Ca-P. Tartrate and trans-aconitate had a slight effect. Therefore, especially citrate-producing strains are important to improve phosphate availability in alkaline soils or in the case of fertilization with rock phosphate. Fe-P and Al-P were mobilized at pH 4 (corresponding to their natural occurrence) by citrate, malate, tartrate, and on a much lower level by gluconate and trans -aconitate. This agrees very well with the carboxylic acid pattern of most efficient phosphate-mobilizing microorganisms (Figure 2a, b, c, d).

## Influence of bacterial inoculation on the growth of maize (*Zea mays*)

According to the data of Table 5, maize plants were in general considerably affected by bacterial inoculation. Plant height obtained with the three *P. fluorescens* strains was significantly different from that of control and the highest score was obtained with strain CE509 (190.2 cm). However, only two of the three strains significantly increased the shoot dry weight, and the best score was that of strain CD511 (83.4 g), followed by strain CB501 (81.0 g). Plant yield was evaluated in terms of mass of grains obtained per plant. It was affected by inoculation with two strains, i.e. CB501 (76.5 g) and CD511 (66.2 g). Shoot and seed phosphorus contents were also influenced by inoculation with all of the strains tested. In gene-

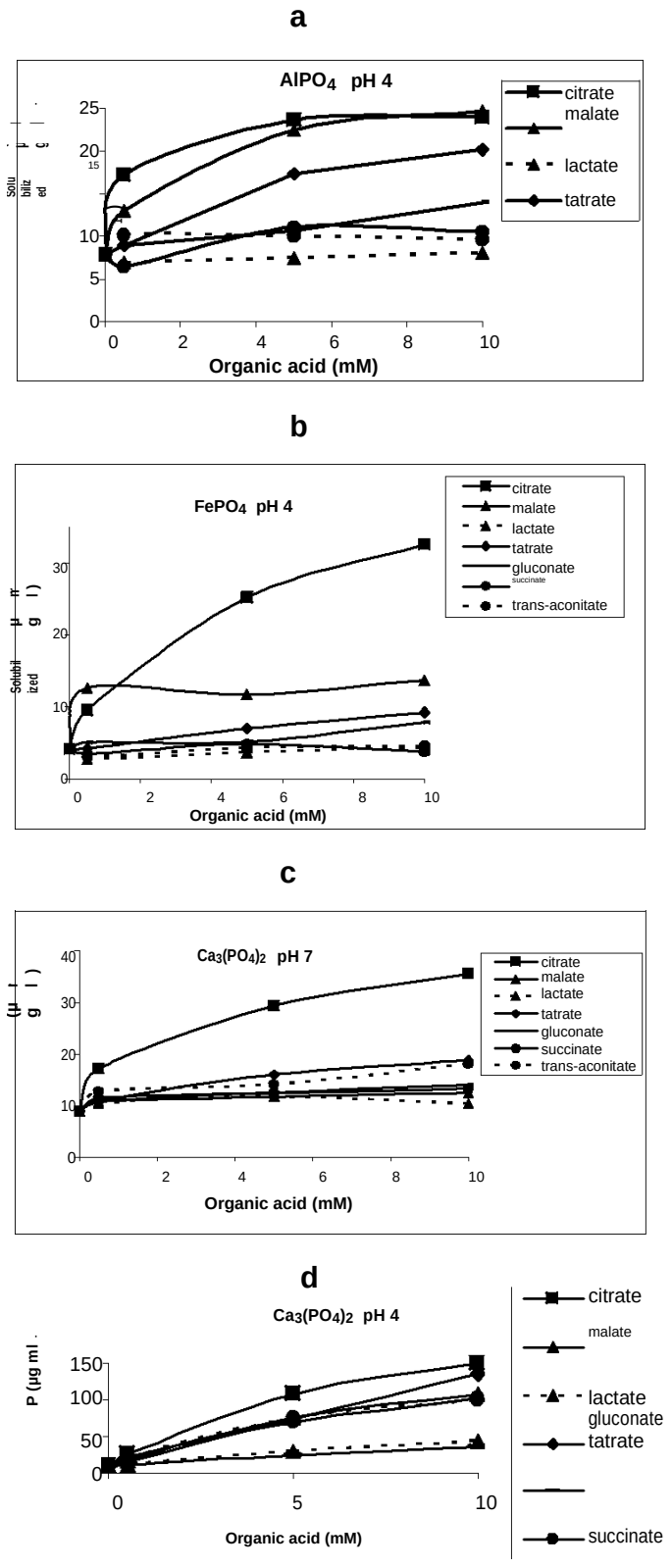


**Figure 2 (a, b, c, d).** Ca-phosphate solubilizing efficiency by different carboxylic acids at pH 7 (2c) corresponding to its natural occurrence and at pH 4 (2d) representing the final pH in microbial cultures and pH of acidic soils. Aluminium (2a) and ferric (2b) phosphates solubilizing efficiency by different carboxylic acids at pH 4 corresponding to their natural occurrence general, all the parameters were significantly affected by at least two strains. According to the plant response to inoculation, over the five parameters studied, *P. fluorescens* CB501 showed significant results in all parameters with a global effect of +37% (Table 6). *P. fluorescens* CE509 and *P. fluorescens* CD511 showed significant results in four of the six parameters, with a global effect of +21.2 and +16.7% for strains CE509 and CD511, res-pectively (Table 6). This confirms the potential of these strains in improving plant growth and/or yield through mineral nutrition.

## DISCUSSION

The main objective of the present study was to evaluate the efficiency of three *P. fluorescens* strains in solubilizing sparingly soluble phosphates in laboratory media and to provide preliminary indications on their effectiveness in improving plant growth and/or yield under nursery-limiting conditions. The first activity of the strains tested was evaluated on agar plates containing sparingly soluble phosphates with dye for better observation (Mehta and Nautiyal, 2001; Gadagi and Tongmin, 2002). This reaction, shown as a halo or clear or yellow (in the present case) zone on the plate, is used to assess the P solubilization activity of these bacterial strains (Fernandez et al., 2007), with the value of the ratio *z/n* as an indicator of the strain efficiency. Thus, the higher the value of the ratio, the greater is the activity of the tested strain. All the strains showed a halo zone at least on agar plates containing sparingly soluble Fe-P, and the halo zone was associated with a pH decrease. This pH decrease was observable through the yellow colour change of the dye (Bromo-Cresol- Green), moving from green (pH greater than 6) to yellow (pH ranging from 5.4 to 3.8).

The test of purity and stability was important in determining whether our strains would be able to mobilize sparingly soluble phosphate found under acidic conditions (Fe-P and Al-P) as in the case of Cameroon soils. Liquid culture experiments involved evaluation of the amount of phosphate released and alteration of pH with time, purification and identification of carboxylic acids at the end of the incubation period in liquid media supplemented with different phosphate types. In general, all the strains showed good aptitude in mobilizing phosphorus from insoluble sources, independently of phosphate type. However, the solubilization rate varied from one medium to another. The solubility of the different phosphates in liquid media decreased in the following order: Ca-P > Al-P > Fe-P. This agrees with results obtained by Ahn (1993) working on inorganic phosphates in tropical soils. Similar results were also obtained by Gadagi and Tongmin (2002) who tested a strain of *Penicillium oxalicum* CBPS- Tsa for $Ca_3(PO_4)_2$, $AlPO_4$ and $FePO_4$ solubilization in Reyes's basal medium. The solubilization of Ca-P was possible by simple acidification of the medium, and acidity undoubtedly contributed to Ca-P solubilization by the carboxylic acids.

**Table 6.** Global effect of phosphate-solubilizing *P. fluorescens* strains on maize under green house conditions.

| Strains | Plant height (%)* | Shoot dry weight (%)* | Plant yield (%)* | Phosphorus uptake (%)* | | Average (%) |
|---|---|---|---|---|---|---|
| | | | | Shoot | Grain | |
| CB501 | +21 | +18.9 | +35.9 | +32.7 | +76.5 | +37 |
| CD511 | +16.7 | +22.5 | +17.6 | +13 | +13.4 | +16.7 |
| CE509 | +22.6 | +5.4 | +4.3 | +19.6 | +53.8 | +21.2 |

(T – C) x $C^{-1}$ x 100 where T = treatment and C = control.

However, acidification could not be the explanation of P mobilization in bacterial cultures containing Fe-P and Al-P. This concurs with the findings of Subba Rao (1982) that acidification of the medium does not seem to be the only mechanism of solubilization, as the ability to reduce the pH in some cases did not correlate with the ability to solubilize mineral phosphates. In this study, Al-P and Fe-P were mobilized at pH 4 corresponding to their natural occurrence by citrate, malate, tartrate, and at much lower levels by gluconate and trans-aconitate. This agrees very well with the carboxylic acid production patterns of the most efficient P- mobilizing bacterial strains (Deubel and Merbach, 2005).

The solubilization of inorganic phosphate in some cases is attributed to the production and release of inorganic acids (Richardson, 2001; Reyes et al., 2001). Because rhizosphere bacteria probably are not able to change the pH of the rhizosphere to any great extent, carboxylic anions are more important as a mechanism for phosphate mobilization. The phosphate mobilizing effect of microorganisms is always a combined effect of pH and carboxylates. Phosphate solubilization occurs by carboxylic acids synthesized and released by microorganisms; this release also decreases pH (Puente et al., 2004; Rodriguez et al., 2006). Previous studies revealed that carboxylic anions are able to replace phosphate from sorption complexes by ligand exchange (Otani et al., 1996; Whitelaw, 2000) and to chelate both iron and aluminium associated with phosphate. Citrate, for instance, is able to release phosphate from goethite (Geelhoed et al., 1999) or amorphous ferric hydroxides (Dye, 1995). Oxalate was also very effective, but was not produced in sufficient amounts by the strains tested. Some previous studies have shown the importance of these carboxylic acids in the process of phosphate solubilization. Ryan et al. (2001) stated that, among the carboxylic acids identified, dicarboxylic (oxalic, tartaric, malic, fumaric, malonic acids) and tricarboxylic (citric) acids are more effective for phosphorus mobilization. According to Illmer and Schinner (1992), gluconic acid may be the most frequent agent of mineral phosphate solubilization. In general, the ability of different carboxylic anions to desorb phosphorus decreases with a decrease in the stability constants of Fe or Al-organic acid complex in the order: citrate > oxalate > malonate/malate > tartrate > lactate > gluconate > acetate > formate (Whitelaw, 2000; Ryan et al. 2001). This result serves to confirm the ability of the strains tested in mobilizing phosphorus from insoluble sources, in particular those producing altogether citrate, malate and tartrate.

In general, plant inoculation with the three *P. fluorescens* strains showed positive effects on the growth and yield of maize. Differences obtained in the fertilizer treatments can be attributed to the nutrients being readily available from the insoluble sources (natural insoluble phosphate or rock phosphate). Thus, inoculation with phosphate-solubilizing *P. fluorescens* strains made more soluble phosphates available to the growing plants. This may be the reason for improved growth and yield of maize plants and could have stimulated microbial growth and activity. Many bacteria (Rodriguez and Fraga, 1999) and fungi (Whitelaw, 2000) are able to improve plant growth by solubilizing sparingly soluble inorganic phosphates in the soil. Moreover, *P. fluorescens* strains are recognized to be good plant growth promoters through the production of growth-stimulating hormones (Schroth and Hancock, 1982) and this may also have affected the growth and yield of the plant.

## Conclusion

The results obtained on agar plates and in liquid media culture showed the real aptitude of the three *P. fluorescens* strains in mobilizing important amounts of phosphorus from the sparingly soluble phosphate sources. Phosphate solubilization occurs by carboxylic acids synthesized and released by microorganisms; this release also decreases pH. Inoculation with phosphate- solubilizing *P. fluorescens* strains made more soluble phosphates available to the growing plants. Such effect indicates the reason for improving the growth and yield of maize plants and stimulating the microbial growth and activity. Moreover, *P. fluorescens* strains may act on plant growth through the production of growth-stimulating hormones. However, the selection of phosphate solubilizing *Pseudomonas* strains as possible inoculation tools for phosphate-deficient soils should focus on the integral interpretation of laboratory assays, greenhouse experiments as well as field trials.

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