

Influence of rhizosphere bacteria of African oil palm (*Elaeis guineensis*) on calcium, iron, and aluminum phosphate in vitro mobilization

Wolfgang Merbach.¹; Henri Fankem.²; Annette Deubel.¹

¹Institute of Agricultural and Nutritional Sciences, Martin-Luther University Halle-Wittenberg, Julius- Kühn- Straße 25, 06112 Halle (Saale), Germany; ²Department of Plant Biology, Biotechnology Laboratory, Faculty of Science, University of Douala, P.O.Box: 24157 Douala, Cameroon;

Contact: tel.0049/345/5522433, fax.0049/345/5527090

wolfgang.merbach@landw.uni-halle.de

Abstract:

In vitro calcium-phosphate mobilization was caused by acidification of the nutrient medium and the production of different carboxylic anions. Under highly buffered soil conditions (neutral or alkaline pH), only citrate can release remarkable amounts of P from $\text{Ca}_3(\text{PO}_4)_2$. Citrate can prevent halo zones on calcium-phosphate agar by precipitation of calcium citrate. This effect reduces the use of such plate tests remarkably!

Down to a pH of 3.5, solubilization of iron and aluminum phosphate cannot be explained by proton release. These phosphates are efficiently mobilized by citrate, malate and tartrate. Common plate tests can fail to detect really effective P solubilizing strains. Therefore, liquid culture or genetic characterization are more reliable methods to elucidate citrate, malate and tartrate producers.

Introduction:

Phosphorus is one of the major factors limiting crop production on many tropical and subtropical soils. Cameroonian soils are mostly acidic causing a strong phosphorus fixation by iron and aluminum. This process decreases also the efficiency of mineral P fertilizers. Rhizosphere microorganisms like mycorrhizal fungi and associative bacteria can improve the use of soil and fertilizer phosphate by plants on soils with strong P fixation (DEUBEL and MERBACH 2005). This study characterized the P mobilizing ability of rhizosphere bacteria isolated in the rhizosphere of African oil palm, one of the most important oil plants in African and Asian countries, with the final aim to select efficient strains as potential biofertilizers.

Methodology:

Bacterial strains were isolated from rhizosphere-soil samples as well as from root-fragment samples of oil palm (*Elaeis guineensis*) cultivated in four provinces of Cameroon (Centre, South, South-West and Littoral). To evaluate the solubilization rate of the obtained isolates, diameter of colonies and halo zones were determined on modified nutrient-agar plates (SUBBA-RAO 1982) containing dye (bromo cresol green) and either $\text{Ca}_3(\text{PO}_4)_2$, $\text{FePO}_4 \cdot 2 \text{H}_2\text{O}$ or $\text{AlPO}_4 \cdot \text{H}_2\text{O}$.

Twenty strains with halo zones not only on calcium phosphate, but particularly on iron and aluminum phosphate were chosen for quantitative tests in liquid culture. Bacteria were grown in 50 ml of Reyes basal medium (REYES *et al.* 1999) with 30 mM of P as $\text{Ca}_3(\text{PO}_4)_2$, $\text{FePO}_4 \cdot 2 \text{H}_2\text{O}$ or $\text{AlPO}_4 \cdot \text{H}_2\text{O}$ at 28 °C on a rotary shaker at 150 rpm. After 3, 5 and 7 days pH and P concentration of the solutions were measured.

For determination of carboxylic acids, nutrient solutions of the most efficient strains were centrifuged and separated into neutral, alkaline and acidic compounds with ion exchange chromatography after 7 days of bacterial growth. The acidic fraction was quantified by HPLC (DEUBEL *et al.* 2000).

In order to determine the influence of pH on the solubility of the three phosphates, $\text{Ca}_3(\text{PO}_4)_2$, $\text{FePO}_4 \cdot 2 \text{H}_2\text{O}$ and $\text{AlPO}_4 \cdot \text{H}_2\text{O}$ were shaken in HCl and NaOH of various concentrations (0-

20 mM) for 90 min. Thereafter, pH and soluble P of the solution were measured. In addition, the mobilizing effect of 0-10 mM of single isolated carboxylates at pH 4 and pH 7 were determined.

Results and Discussion:

All strains released significant amounts of P from $\text{Ca}_3(\text{PO}_4)_2$ in a range of 1 up to 10 mM. Interestingly, the most efficient strain showed large halo zones on iron and aluminum phosphate, but did not form zones on calcium-phosphate agar plates. Calcium-phosphate solubilization was combined with a pH decrease in nutrient solution.

$\text{AlPO}_4 \cdot \text{H}_2\text{O}$ was also mobilized by all strains (up to 3 mM P). Seventeen strains increased the solubility of $\text{FePO}_4 \cdot 2 \text{H}_2\text{O}$ significantly (up to 2 mM P). The pH of nutrient solution decreased more than in treatments with calcium phosphate (down to 4.0-3.5 in single strains), but without correlation with P release.

The most efficient $\text{Ca}_3(\text{PO}_4)_2$ -mobilizing strains produced large amounts of citrate and tartrate and some malate. Most important carboxylates in iron and aluminum solubilizing strains were tartrate and malate. In some strains, also xylonate and gluconate was observed as well as small amounts of citrate, fumarate and trans-aconitate. Carboxylate production could be underestimated by precipitation of insoluble metal compounds.

In additional tests, the solubility of calcium phosphate increased exponentially with decreasing pH. The solubility of iron phosphate decreased with a pH decrease down to 4.5-3.5. Aluminum phosphate showed the lowest solubility between pH 5.5 and 4.5. At pH 3.5 it was comparable with pH 7. Hence, acidification cannot be the explanation for P mobilization in bacterial culture in the last two cases.

At a low pH, all identified carboxylic acids solubilized $\text{Ca}_3(\text{PO}_4)_2$ with different efficiency. This explains P-release in *in vitro* cultures. However, sparingly soluble calcium phosphates occur only in neutral and alkaline soils. A strong pH decrease is impossible under well-buffered soil conditions. At pH 7, only citrate solubilized remarkable amounts of calcium phosphate. Tartrate and trans-aconitate had a slight effect. Therefore, especially citrate producing microorganisms should be selected to improve P availability on alkaline soils or in case of fertilization with rock phosphate.

Iron and aluminum phosphate were mobilized at pH 4 (corresponding to their natural occurrence) by citrate, malate, tartrate, on a much lower level by gluconate and trans-aconitate. These findings agree very well with the carboxylic-acid pattern of most efficient P-mobilizing strains.

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