

## Development of cheap, eco-friendly and sustainable amendments to improve plant growth

Phosphorus (P) is an essential nutrient for the growth and development of plants. Its limited availability due to the low solubility, often limits crop production. Therefore, soluble phosphate fertilizers are commonly applied to achieve maximum crop productivity. However, most of the soluble P applied is rapidly converted to unavailable forms making necessary repeated applications that greatly exceeds plant needs in P and generates environmental problems. Moreover, the traditional production of such phosphate fertilizers is based on chemical processing of insoluble mineral rock phosphate (RP), which includes an energy intensive treatment with sulfuric acid at high temperature.

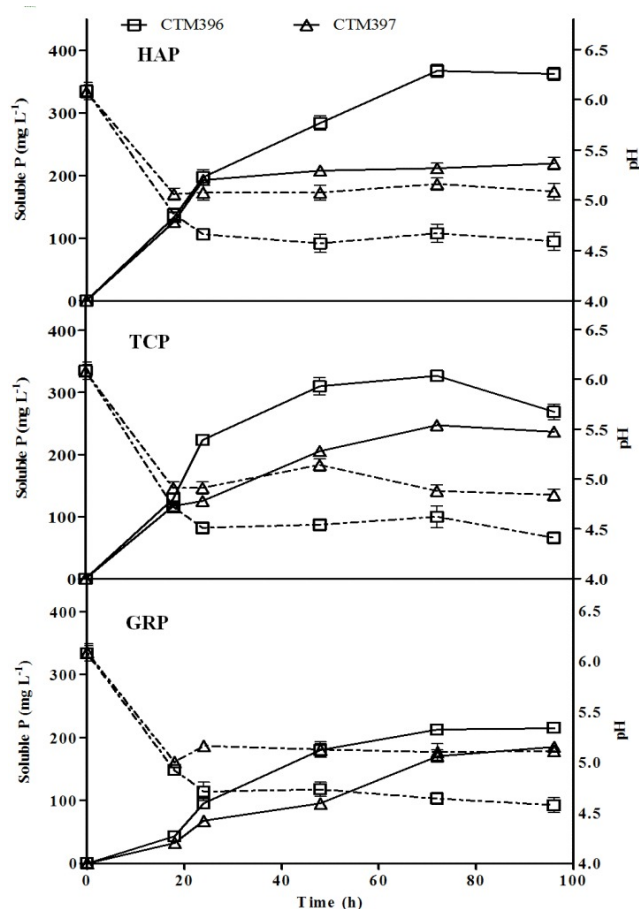


Fig. 1. Evolution of soluble P (continuous line) and pH (dotted line) in the growth medium of *Streptomyces* sp. CTM396 (square) and CTM397 (triangle) grown at 30 °C on NBRIP media containing 5 g/L of HAP, TCP and GRP.

This process has become an environmentally undesirable and costly affair. Furthermore, the current developments in sustainability require a strong reduction in chemical fertilizers inputs and their replacement by cheaper, eco-friendly and sustainable practices. For instance, insoluble inorganic phosphates including RP can be transformed into soluble forms available to plant roots by the action of some soil microorganisms termed as phosphate solubilizing microorganisms (PSM). Mineral phosphate solubilisation (MPS) is an essential plant growth-promoting ability via which PSM have found extensive applications in agriculture as bio-inoculants.

In the present study, a total of 128 isolates of actinomycetes which are Gram-positive soil-inhabiting filamentous bacteria were taken from soil samples collected near a rock phosphate processing site belonging to the Tunisian Chemical Group of Sfax (Tunisia), and were screened for MPS ability. A significant MPS activity was observed for 30 isolates on various inorganic phosphate sources (like Gafsa rock phosphate "GRP"; hydroxyapatite "HAP" and tricalcium phosphate "HAP") when grown in the National Botanical Research Institute's phosphate broth (a growth medium allowing easy selection of PSM). The selected strains namely CTM396 and CTM397, which showed the highest MPS abilities, were identified by molecular approaches as members of the bacterial genus *Streptomyces*.

Their MPS activity was proved to be concomitant with a drop in pH of culture broth due to the secretion of gluconic acid (GA), a low molecular weight organic acid, which via its carboxyl group chelates the cations bound to phosphate, or by the liberation of  $H^+$  protons, thereby converting it into soluble forms available for plants. This was correlated with the simultaneous detection by Polymerase Chain Reaction (PCR) of the genes *gdh* (encoding the glucose dehydrogenase GDH, enzyme responsible for GA production by direct oxidation pathway of glucose) and *pqq* (involved in biosynthesis of the pyrroloquinoline quinone cofactor needed for the GDH enzyme catalytic activity), as well as the highlighting of GDH enzyme activity, for the first time in a *Streptomyces* sp. strain producing GA. Furthermore, the addition of humic acids (compounds known to exert a number of essential physical, chemical, and biological functions to sustain soil fertility and promote plant growth) at 0.05% of proved to have a stimulatory effect on the growth and the ability of CTM396 to solubilize Gafsa rock phosphate.

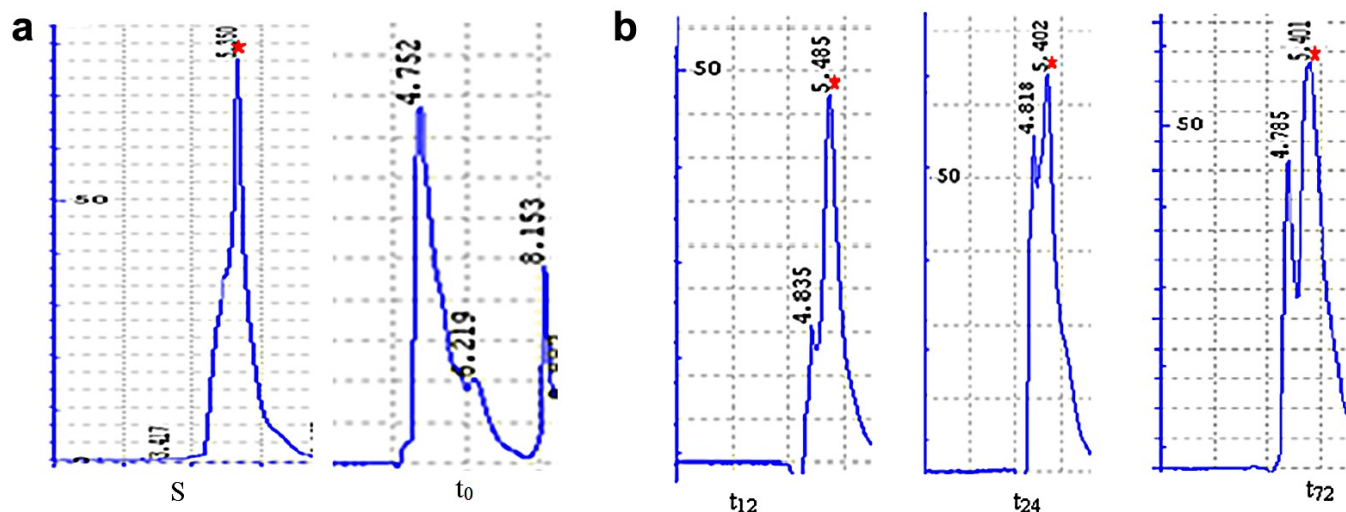


Fig. 2. Identification by HPLC of gluconic acid (GA) in the culture filtrates of *Streptomyces* sp. CTM396 grown at 30 °C on NBRIP supplemented with 5 g/L of GRP. The chromatographic separations show A260 (y axis) versus time (minutes). GA showed retention times of about 5.350 (S). GA at 50 g/l in 0.05 M NaH<sub>2</sub>PO<sub>4</sub> (pH = 6.5) was added to the culture filtrate prior to bacterial incubation and shown as t<sub>0</sub> (a). Identification by HPLC of gluconic acid and P released in the culture filtrates of *Streptomyces* sp. CTM396 (b) grown at 30 °C in NBRIP supplemented with 5 g/L of GRP. t<sub>12</sub>, t<sub>24</sub>, t<sub>72</sub> correspond to filtrates from culture of CTM396 grown for 12, 24, and 72 h. The peaks corresponding to GA are indicated by asterisks.

In summary, according to this study, it is possible to use humic acids and rock phosphate in association with spores of ad hoc *Streptomyces* strains (exhibiting MPS ability) as natural, eco-friendly, sustainable and efficient amendments to improve plant growth with no need of costly and pollutant transformation of natural rock phosphate.

## Publication

[Mineral phosphate solubilization by \*Streptomyces\* sp. CTM396 involves the excretion of gluconic acid and is stimulated by humic acids.](#)

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*FEMS Microbiol Lett.* 2015 Mar