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Short communication

# Solubilization of phosphates *in vitro* by *Aspergillus* spp. and *Penicillium* spp.

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ABSTRACT

Phosphorus (P) is one of the most important nutrients for plant development and in most Brazilian soils the content of this element is low and relatively available to plants. Phosphate-solubilizing microorganisms play an important role in supplying P to plants, because of their ability to provide insoluble phosphates, added or existing in the soil, by the processes of acidification, chelation and ion-exchange reactions. The objective of this study was to evaluate the capacity and potential of ten fungal isolates in solubilizing simple superphosphate (SSP) and mono-ammonium phosphate (MAP) *in vitro*, in four periods (1st, 4th, 7th and 10th days after inoculation). It was found that 90% of these isolates showed potential for solubilization of SSP and MAP on the seventh day of evaluation, with average values of 23% and 22% higher than control, respectively, with a reduction thereafter. This reduction can be attributed to the increase in fungal biomass, which results in enhanced uptake of soluble phosphate for growth. All isolates, except for the PSF 94, solubilized the two sources of phosphate on the 7th day, but the isolated PSF 28 in MAP, and PSF 220 in SSP, stood out from others by having the highest values of soluble P (84 and 56 $\mu g\,ml^{-1}$, respectively). This is the first reported solubilization of single superphosphate and mono-ammonium phosphate *in vitro* by the *Aspergillus* and *Penicillium* species, demonstrating that these fungi can serve as phosphate-solubilizing these P sources, contributing to a better use of the SSP and MAP and reducing the cost of agricultural inputs and the impact caused by excess phosphorus.



## 1. Introduction

Among the essential elements, phosphorus (P), followed by nitrogen (N), has a prominent place for living beings, in view of its structural and functional performance as well as energy transfer (Sharpley, 1995; Bissani et al., 2008). In general, Brazilian soils have low phosphorus appearance, being the soluble phosphorus content very low (0.03 mg P $kg^{-1}$), requiring the application of phosphates in amounts far above the demands of the plants, due to the great reactivity and high retention rate of their anions to various constituents of soil (Mendes and Reis Júnior, 2003). Thus, the soluble forms are easily precipitated in insoluble complexes and are not efficiently absorbed by plants. This way, in order to overcome this obstacle, excessive doses of P are applied to achieve the production and economic return (Vassilev and Vassileva, 2003).

On the other hand, several soil microorganisms, including bacteria, fungi and actinomycetes, have the ability to solubilize insoluble phosphates, converting them into soluble forms that are available to plants through different mechanisms, such as acidification, chelation and ion-exchange reactions, especially in this case, the production of acids (Sahu and Jana, 2000; Whitelaw, 2000). Due to the fact that these microorganisms are present in most soils, the solubilization of P through them can be a lower cost alternative to production in agriculture (Rajan et al., 1996; Mendes and Reis Júnior, 2003).

One of the areas that require the most nutrients is fruit production, which by its intensive nature, consumes with fertilizers nearly 10% of total production costs (Albuquerque et al., 2009) which justifies the study of alternative practices that enable lower costs, without prejudice to productivity and product quality as well as the environment.

Among the cultivated fruit in the Submédio region of the Vale do São Francisco (VSF), the vine (*Vitis vinifera* L.) is one of the most important due to the generation of employment and income, contributing with 99% (80,000 tons) of exports of grapes in the country (Silva et al., 2009). Besides the production of table grapes, the areas of grapes for wine and juice are expanding, justifying the need for alternatives to the efficient use of phosphate fertilizers. From these, mono-ammonium phosphate (MAP) and single superphosphate (SSP) are widely used in the culture of the vine in the VSF

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mainly because they contain, in addition to P, nitrogen (MAP) and calcium and sulfur (SSP).

Although these phosphated sources are water soluble (44% MAP and 16% SSP water soluble), part of P can become adsorbed to the surface of colloids or converted into very poorly soluble compounds, thus studies in order to optimize the use of phosphated fertilizers added to soil through solubilization by fungi is desirable, contributing to the establishment of a sustainable agricultural system, based on greater efficiency in the use of non-renewable natural sources.

The solubilization of P by fungi has been reported, especially in the *Aspergillus* species of the niger group (*Aspergillus aculateus*, *Aspergillus awamori*, *Aspergillus niger* and *Aspergillus tubingensis*) (Achal et al., 2007; Ahuja et al., 2007; Reddy et al., 2002; Vassilev et al., 2007) and some species of *Penicillium* (Oliveira et al., 2008; Saber et al., 2009; Vyas et al., 2007; Xiao et al., 2008).

The increase in availability of P by the fungi may vary due to the source and fungal isolate. Vyas et al. (2007) reported that the rate of solubilized phosphate by *Eupenicillium parvum* ranged from 120.8 to 213.7 $\mu g\,ml^{-1}$, averaging 121 to 214% higher than the control. On the other hand, Gupta et al. (2010), assessing the ability of *Aspergillus* sp. as to the solubilization of rock phosphate (India), found variation from 45.2 to 54.4 $\mu g\,ml^{-1}$ of soluble P, an increase from 63 to 105% compared to control.

Some studies have reported the attempt to increase the availability of inorganic phosphate by inoculation with phosphate-solubilizing fungi (PSF), being the majority of experiments conducted in laboratories and greenhouses. Saber et al. (2009) evaluated *A. niger* and *Penicillium* sp. as inoculants in *Vigna radiata* (L.) R. Wilczek plants and found an increase of soluble P in the fertilized rhizosphere with tricalcium phosphate (211 $\mu g\,ml^{-1}$), aluminum phosphate (104 $\mu g\,ml^{-1}$), rock phosphate (99 $\mu g\,ml^{-1}$), sodium phytate (89 $\mu g\,ml^{-1}$) and iron phosphate (33 $\mu g\,ml^{-1}$). Recently, Kapri and Tewari (2010) reported an increase in the dry weight of the aerial part (22–33%) and of the root (35–60%) of chickpea plants in the presence of *Trichoderma* sp. in soil fertilized with tricalcium phosphate, when compared to controls without inoculation of the fungus, demonstrating the potential application of these microorganisms.

Studies indicate that the PSF may constitute a viable alternative to maximize the use of phosphorus. Although the use of PSF is extremely important or more relevant in natural phosphate sources, information about the solubilization in MAP and SSP do not exist and even being very soluble, PSF may contribute further to their solubilizations. Thus, the objective of this study was to evaluate the capacity and potential of ten fungal isolates, derived from the culture of the vine in solubilizing mono-ammonium phosphate and single superphosphate *in vitro*, analyzing the influence of the incubation period of such activity.

## 2. Materials and methods

Ten specimens of PSF, eight of *Aspergillus* (PSF 9, 28, 39, 57, 145, 198, 212 and 220) and two of *Penicillium* (PSF 94 and 169), were isolated from rhizospheric soil of the vine (*V. vinifera* L. cv. Cabernet Sauvignon) in the region of the Submédio of the Vale of São Francisco (Farm Planaltina, ViniBrasil), Petrolina, Brazil (08°59′49″S, 40°16′19″W) using the technique of suspension in series (1:1000, v/v) of soil.

These PSF were grown in erlenmeyers containing 50 ml of GL medium (Sylvester-Bradley et al., 1982) liquid supplemented with 0.52 g 50 $ml^{-1}$ of mono-ammonium phosphate (MAP) or 1.39 g 50 $ml^{-1}$ of single superphosphate (SSP), corresponding to 0.25 g $P_2O_5$ 50 $ml^{-1}$, with medium pH adjusted to 6.5. One milliliter of spore suspension of each PSF specimen, representing about $10^7$ spores $ml^{-1}$, was added as inoculum. The erlenmeyers were incubated in a BOD (biochemical oxygen demand) at 30 °C for a period of 10 days. Erlenmeyer flasks with the same medium, without inoculation, corresponding to the control group were maintained.

Evaluations of P in the solution were carried out after 1 (T1), 4 (T2), 7 (T3) and 10 (T4) days after inoculation, withdrawing 5 ml of the culture of each flask. The cultures were centrifuged at 10,000 × *g* for 5 min and the supernatant was filtered (Whatman paper No. 40). Phosphorus in the solution (filtrated) was determined by spectrophotometry (660 nm) according to procedure described by Tedesco et al. (1995). The pH of the supernatant was determined by pH meter and the number of spores was quantified in a Neubauer chamber. At the end of the incubation period, the mycelium of each treatment was washed with distilled water and dried at 70 °C for 72 h or until constant weight for estimation of the dry biomass.

The experimental design was the completely randomized type with the factorial of 11 (10 isolated and control) × 4 periods (T1, T2, T3 and T4) in three repetitions. The variables analyzed were soluble P, pH and number of spores. Analyses of simple correlations were made between soluble P, pH and number of spores.

Data were submitted to analysis of variance (ANOVA) and averages were compared by the Tukey test at 5% probability, using the program Statistica 5.0 (Statsoft, 1997).

The data of soluble P was used to calculate the increase (Weber et al., 2004) provided by the PSF, using the formula $100[(X - Y)/Y]$, where $X$ represents the inoculated treatment with the PSF and $Y$, control treatment.

## 3. Results and discussion

There was an effect of the factors and the interaction between isolates and evaluation periods for all analyzed variables, for both sources of phosphate.

On the first and fourth days of assessment (T1 and T2), there was no difference between control treatment and the isolates; the only isolate that showed small potential for solubilization in both sources of P and remained statistically equal to control in T3 and T4 was PSF 94.

It was found that 90% of the isolates showed potential for solubilization of single superphosphate and mono-ammonium phosphate on the seventh day of evaluation (T3), with average values 23% and 22% higher than control, respectively, with a decrease after this period of time (Tables 1 and 2). According to Barroso and Nahas (2008), this reduction in the availability of P is related to the increase in fungal development, which leads to enhanced uptake of soluble phosphate by the fungus for its own growth, both vegetative and reproductive. On the 10th day (T4) the number of spores was higher (Tables 1 and 2), making it probable that the PSF tested have also mobilized part of the source of phosphate in its mycelium, contributing to the decrease in the amount of soluble P. Clearly, as seen by the results of PSF 94, which presented the greatest number of spores, in contrast, the concentration of soluble P did not differ from control. However, PSF 39 (MAP and SSP) and PSF 220 (SSP) also had higher soluble P in T4, which may be related to the metabolism of growth of these isolates.

All the isolates, except PSF 94, solubilized P from the two phosphate sources in T3, although the isolated PSF 28 and FSP 220 stood out from others by having the highest values of soluble P in MAP and SSP (84 and 56 $\mu g\,ml^{-1}$), respectively, being the rates of increase in comparison to control 29% and 44%. Inoculation of PSF in experiments which took place in the greenhouse or field could reduce